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REPORT

Following the implementation of the New Education Policy,(86) the NCERT/RCE has given its attention to improve the quality of Science & Mathematics Education. As a part of this scheme, this college is organising and conducting orientation programmes for teachers of different establishments. Recently Mr.T.K.Bal Subramaniam, Director, Establishment (Welfare), Indian Railway Board, New Delhi, made a formal request to Dr.A.K.Gopalan, Director, NCERT to organise an Orientation Programme for the Post-graduate teachers of the Railway Board Schools in Biology. This responsibility in turn was taken up by Regional College of Education, Bhubaneswar and conducted a Training Programme for 10 days from May 20 to May 30,1992.

The participants for this programme was drawn from all over India. In all there were 29 participants, 10 from West Bengal, 6 from Madhya Pradesh, 4 from Uttar Pradesh, 2 each from Assam and Orissa and 1 each from the States of Bihar, Rajasthan, Andhra Pradesh, Tamil Nadu and Maharastra.

The programme was inaugurated on May 20,1992 by Dr.K.C. Panda, Principal, R.C.E.Bhubaneswar. In his inaugural address Dr. Panda stressed the need for improving the competency and efficiency of teachers in transacting the curriculum in classroom and the importance of the present Orientation Programme. Further he called upon the teachers to change the method of evaluation in view of the new demands of NEP. Dr. Panda assured, to all those present, to place the facilities and the services of this college for any future training/orientation programme if a specific request is made. Mr. Subramaniam who also spoke on the occassion and urged the participating teachers to take full advantage of the programme and the opportunity that is provided, through active participation in all the deliberations and other activities of the programme.

It may not be out of place to mention that the teachers who were present were exposed for the first time to this type of Orientation Programme after their recruitment by the Railway Board. Keeping in view the short duration of the programme and also different curricula that the teachers follow in their respective schools, the faculty members of this college in consultation with the participants identified the specific areas to be dealt with during the programme.

The main objective of the programme was to enrich, update and reinforce the content knowledge of the participants and provide information on new concepts and latest development in the field in which they have very little exposure. Hence it was decided to give stress on areas like Cell Biology, Genetics, Evolution, Animal & Plant Anatomy and Physiology, Animal Embryology, Mycology and Plant Pathology, Environmental Biolog Biotechnology, Genetic Engineering, and few lectures on Pedago The Department also arranged extension lectures by eminent Professors and Scientists. All the lectures were supported by appropriate laboratory work.

At the end of the programme, the participants were asked to evaluate the programme. From the information obtained from the participants and its analysis thereafter revealed the following facts:

1. 85% of the participants were of the view that the open discussion adopted by the resource persons were quite satisfactory and there was proper motivation while introducing the subject matter.
2. 80% were of the opinion that the presentation of topics by Resource Persons were clear, definite and useful.
3. The practicals were relevant and provided an opportunity for the development of skills.

4. There were mixed reactions as far as the interaction between resource persons and participants was concerned.

5. 45% believed that the interaction was good whereas the rest felt it was average.

The participants have expressed their views about the strong and weak points of the programme. Some of the common points made out are:

STRONG POINTS:
(a) The programme was well organised.
(b) The resource persons were highly qualified and experienced.
(c) Both theory lectures and practicals were of high standard.

WEAK POINTS:
(a) The duration of the programme was short.
(b) The Library facilities were inadequate.
(c) Cyclostyled materials of lectures was not provided immediately.

The participants have also given some suggestions for the improvement of the programme in future.

1. The duration of the programme should be enhanced.
2. The programme schedule should be strictly followed.
3. Techniques and pedagogical aspects of teaching at +2 level should be appropriately highlighted.

The faculty members of the Biology Department of this college are of the unanimous view that the involvement and performance of the participants, on the whole, was very good and encouraging.

I express my deep sense of gratitude to Prof.K.C.Panda, Principal, R.C.E.Bhubaneswar for his valuable suggestions and guidance, both academically and administratively, while conducting this programme. My sincere thanks are also due to all the members of staff, academic and non-academic, of the department for the help rendered by them.

DYNAMICS OF ECOSYSTEM

Compiled by Dr.P.K.Durani,
Reader in Botany,
R.C.E.,Bhubaneswar.

Ecological relationships are manifested not in vacuum but inphysico-chemial settings, sets of nonliving a abiotic environmental substances and gradients. These include basic inorganic elements compounds such as H_2O and CO_2, Ca, and O_2 etc. and an array of organic compounds, the by products of organism activity or death. It is against this abiotic backdrop that the biotic components, plants, animals and microbes in a fundamentally energy dependdant fashion, the abiotic physico-chemical environment and the biotic assemblage of plants, animals,and microbes comprise a system - known as 'ecosystem'.

a) Physical environment: Planet earth is one of the nine planets that revolves around an incandescent ball of gaseous star-the Sun. The earth has

   i) an atmosphere of its own with O_2, N_2 and Co_2 as life sustaining components.,

   ii) a temperature which can support life,

   iii) an input of tremendous quantities of energy from sun and

   iv) the main stay of the basic life sustaining energy conversion reaction - PHOTOSYNTHESIS which is most important single factor influencing living system.

The earth may be regarded as a 'system' within the 'systems'. The various systems that control the life on earth are a) abiotic system or physical environment comprised of weather and climate, rocks and soil and (b) biotic components comprised of plants, animals and microbes. All the Abiotic components are highly inter-related and interdependent on the biotic component of the environment.

Light, temperature, rainfall and wind are the components of climate.

- light affects photosynthesis in plants and reproduction in living systems.
- Temperature controls bio-chemical processes, transpiration and evaporation from plant and animal surfaces. We have warm blooded animals (the birds and mamals) with wide range of temperature tolerances and cold blooded animals (fishes) with narrow range of tolerances. Thus we have Eurythermal (widerange of temperature tolerances)and stenothermal (narrow range of temperature tolerances) organisms.

S.D.P.

- Rainfall and other forms of water like snow, ice, hail, frost, dew (collectively known as precipitation) control the abundance, composition, diversity, distribution and structure of all living organisms. Thus we have desert vegetation, forest vegetation, green lands, thorns and bushes all determined by amount and distributionof precipitation per year.

- The direction and intensity of wind controls size and shape of plants, evapo-transpiration, translocation of particulate matter like dust, sand and pollen, and translocation of water vapours from soil and living organisms and waste gases from industrial installations.

Plants and animals are also greatly influenced by physiographic factors. Physiographic factors include attitude, latitude, longitude and the steepness of the region, nature of the underlying rocks and the extent of weathering and erosion. It should be understood that physiographic factors have implication on temperature, and atmospheric pressure, plant and animal population including man (altitudinal effect), soil building (weathering effect) and the stability of soil surface, topography etc.(Nature of rock effect).

Soil factor includes the nature and type of soil. Soil texture, porosity, aeration, water holding capacity, micro-organisms and 'humus' content determine the soil factors.

Soil 'Humus' deserves a special mention. Dead animals and plants (litter) under the influence of several microscopic organisms (Bacteria, fungi, protozoa and macroorganisms (earth worms, ants, white ants etc.) break down into amorphous black particulate matter known as 'humus' that ultimately mingles and integrates with soil particles. Further disintegration of 'Humus' releases all the minerals tapped in it thus enriching and replenishing the soil with the minerals removed by plants when alive. Humus content also determines physical (Porosity, water holding capacity, aeration etc.) and chemical (Acidity, alkalinity, mineral and nutrient content etc.) characteristic in the soil.

b) Biological system:- Biological system consists of Plants, animals and microbes.

Biological components consists of diverse types of plants, animals and microbes ranging from simple celled simple microscopic organisms like Amoeba, Chlorella, Bacteria, to highly complex multi-cellular forms like trees, herbs and shrubs, several algae, edible and poisonous mushrooms, fish, toads, flesh eating animals like lion and tiger, mamoth elephants and above all man himself. Some of these living organisms are autotrophs (self feeding) like trees of the forests, grasses of grassland, algae of ponds and all those which possess that unique green pigment which we call chlorophyll with the help of which solar energy is trapped and simple abiotic or physical components of environment like water and carbon dioxide are converted into complex forms like Glucose, Carbohydrates, proteins fats etc. or 'Food'.

Part of the food manufactured by the green plants by process of photosynthesis is utilized by the manufacturer (green plant) and part of it is shared by all other types of organisms which lack the Chlorophyll and are termed as Heterotrophs.

All living organisms with similar habitat requirements tend to occur together and occupy specific areas inthe habitat. Such specific areas are known as 'Niches'. Variety of relationship have evolved amongst the living organisms which include competition between 'similars' and 'disimilars', predator, Symbiosis, Parasitism, Commesalism. Several examples can be cited for each such relationship - like competition between two grass plants for light; hawk (Predator) and Snake (Prey); Round worms (a Parasite) -man (host) lichen (a symbiotic association between algae and fungus for mutual benefit). Man, himself, is a best example to illustrate the varied type of relationship it develops with physical and biological environment. Inspite of the fact that man is capable of manipulating almost all factors in the environment possesses variety of relationships ranging from equal competition to symbiosis.

No organism including man lives in isolation: All living organisms develop meaningful interrelationship with other organisms on one hand and physical environment on the other hand thus giving rise to yet another complicated type of interrelationship called as Ecosystem Interrelationship.

2. Ecosystem Interrelationship

Ecosystem interrelationship involves transfer of food and energy from one living component to another. This relationship starts with the trapping of Solar energy by green plants (called as producers) by a process of photosynthesis and its subsequent transfer to herbivores like (Rabit) which feed directly on green plants (herbivory) and then to Carnivores (like lion, tiger, snake) which feed on the herbivores (Carnivory) thus setting a chain of food (Energy) transfer from one feeding level to another level in a systematic chain like fashion known as food chain or trophic chain with well marked levels known as trophic levels. At each trophic, level some energy is lost by way of respiration (metabolism). Therefore, the amount of energy which is passed on to next trophic level decreases. As such no energy is left for recirculation at the last trophic level. Unidirectional and non-cyclic flow of energy is fundamental characteristic of x all Ecosystem. Apart from Autotroph-Heterotroph; Producer - Consumer or producer - Herbivore-Carnivore Food chain, there exists another type of food chain in the ecosystem which is called as the Detritus food chain which involves the action of several forms of macro-organisms (Bacteria, fungi, Protozoa etc) collectively called as the decomposers on dead bodies of producers, herbivores and carnivores. These decay organisms act on dead bodies to nourish themselves and release into the system what is excess for them and thus cause recycling of inorganic nutrients to renew plant and animal population.

Ecosystem may be defined as any system which involves the interaction between the living and nonlivihg components. This leads to flow of energy that leads to clearly defined trophic structure, biotic diversity and exchange of nutrients. Within this system of relationships (Ecosystems), several food chains are linked together thus forming food webs. Some examples of food chain and food webs may be cited.

On land simple food chains-

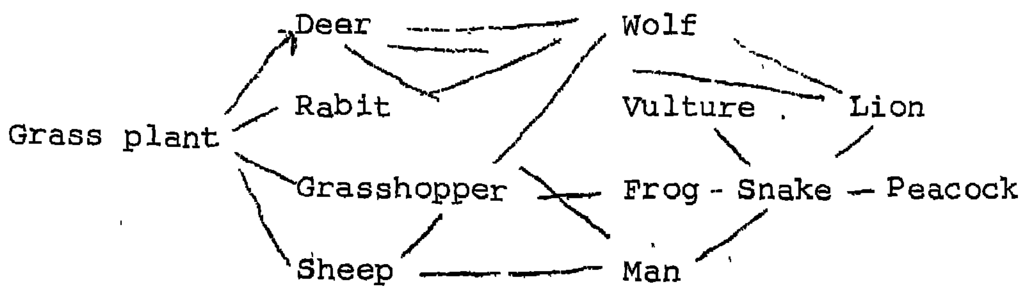
(Green) Producer - Grasshopper (Herbivore) - Frog - Snake - Peacock.

1. Grassplant - Rabit - Wolf
2. Green plant - Dear - Lion
3. Grass plant - Goat - Man
4. Grass plant (Wheat) - Man

In water:-

1. Green algae - Insect larvae - Small fish - Large fish
2. Phytoplankton (Green algae) - Crestaceals - Zooplanktons - Small fish - large fish - man

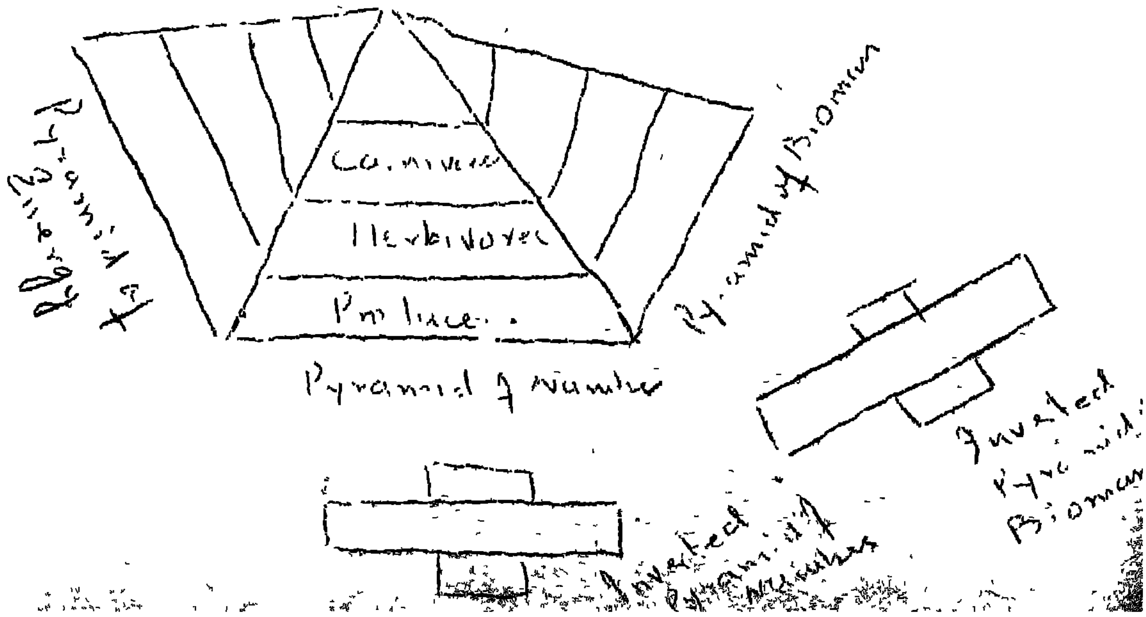
Food Web



In a balanced ecosystem, competition is such as to keep each section of population at a level at which none is threatned and each can retain its individuality. It, therefore, follows that at each trophic level number of organisms that can be accommodated is optimum. This number decreases as the trophic level increases from producer level to top carnivore level. Thus resulting in a pyramid type of model known as "Pyramid of numbers" which under certain conditions may be inverted also. For example a single large tree sheltering a large number of fruit eating birds and a few birds of prey.

The total mass of living matter or biomass which can be supported at a given trophic level at a particular time also gets less in natural system as the trophic level increases resulting in a Pyramid of Biomass which at times may be inverted.

The energy content under all circumstances decrease from lower to higher trophic levels resulting always in an erect "Pyramid of Energy". The three types of Pyramids together are known as Ecological Pyramids. Digramatically represented as follows:

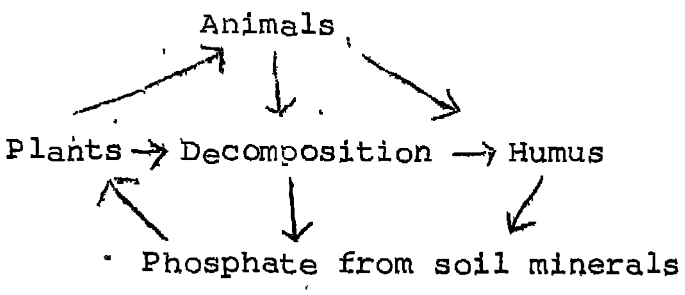


Minerals are essential for Ecosystem Functioning:

Apart from solar radiation, there are about 22 elements needed to build organic substances by living organisms especially plants. Some of these are needed in large quantities like C,H,O,N, P etc. and are known as Macronutrients. Some of these are readily available in nature in large quantities as C,O,N. Several elements though needed in extremely small quantities (Micro-nutrients) are scarcely available in nature (Co, Zn, Boron, Manganeese etc.) Majority of these nutrients are made available by series of cyclic arrangements in nature. The cyclic movements of the nutrients in the ecosystem constitute yet another important aspect of Ecosystem inter-relationships and referred to as Bio-geo-chemical cycles: They are categorised into

1) Hydrological cycle or water cycle involves the exchange of water between oceans, land masses and as a result of evapo-transpiration and atmosphere as a result of precipitation. The balance between evapo-transpiration and precipitation in a given region determines its fauna and flora.

2) Sedimentary cycles are exemplified by cycles like phosphorus and sulphur. A simplified diagram of phosphorus cycle is shown as follows:



As a constituent of Protoplasm (nucleic Acid; phospholipids and numerous phosphorylated compounds), phosphorus is one of nutrients of major importance to biological systems but of all the nutrients, phosphorus, perhaps, is the most rare nutrients available only through the decomposition of phosphate rocks and humus.

Gaseous cycles are exemplified by carbon, nitrogen and oxygen cycles. All these have the atmosphere as the major reservoir. These cycles show little or no permanent change in the distribution and abundance of the elements.

Nitrogen cycle involves the convercion of atmospheric N_2 into nitrites and nitrates (nitrification) by nitrite and nitrite bacteria). Ammonia (Ammonification) by Ammonifying bacteria and conversion of Ammonia, Nitrates and Nitrites into gaseous N_2 (Denitrification) by denitrifying Bacteria, several types of plants especially members of pea family (Leguminosae) directly convert atmospheric N_2 into nitrogen compounds (subsequently incorporated with protoplasm) with the help of symbiotic bacteria like Rhizobium (recollect root modules of Pea) - a process known as Nitrogen fixation. Blue green algae are important in this regard. What appears to be a simple cycle is in fact more extensive, complicated and ordered. It is also highly specific in that certain organisms are able to act only in certain phases of the cycle.

Carbon Cycle is perhaps the simplest of all nutrient cycles and is essentially perfect cycle in that carbon is returned to the environment about as fast as it is removed. This basic movement of carbon is from the atmospheric reservoir to producers to consumers and from both these groups to decomposers and thence back to reservoir. It should, however be mentioned that Oceans hold more than 50 times greater Co_2 than the atmosphere and there is a constant exchange of Co_2 from atmosphere to the oceanic reservoir. In past few decades increased use and incomplete combustion of fossil fuels has resulted in detectable increase of atmospheric carbon. The prediction is for a 25% increase in atmospheric Co_2 by the end of century. What the long term effects will be of such an increase or of further increase is a matter considerable and immediate concern which needs to be discussed in detail.

In the natural undisturbed state these cycles will continue to operate continuously so that a sufficiency of the malericts they circulate is ensured.

Nature has enough to fulfil man's need. But at presents man works for his 'greed'. He has converted forest of magnificient trees into forests of 'Concrete'. Man has constricted waterways and erected dams and reservoirs. He has chocked the streams and filled all possible water bodies with any fish that can swim. In the name of cultural and industrial revolution man has invented polluted culture, polluted poltical system and polluted environment. We are slapping the nature so mercilessly and robbing its natural resources so brutally that we have current idiom "the world's ills involve three 'P's -'Pollution, Population, and Poverty'. It is imperative, therefore, for a teacher and teacher-educator to understand these problems and bring about the message in clear and in an unambiguous tone that constant misuse of nature will 'boomrang'. That pollution has been a concomitant of man's existence as have the effects of population growth; that the status of these problems is to be determined by their urgency and immediacy. A swallen stream, for exampe, does not await the training of Engineers who in turn construct dams and diverge waterways. It warrants an immediate convergence of sandbags on the stream banks. With the flood crest passed, the sandbags may remain for sometime as a grim reminders of a torturous experience but most likely soon begin gradually to fall into disorder . The immediate problem of the flood is resolved, but the long-range problem of managing water resource is not, for it could happen again. What has this to do with the ecologist ? Simply that immediate problems can be solved, like stopping the discharge of industrial acid into a stream that serves as a source of potable water for community downstream. The long-range problems of learning how to cope with the by products of man's biology and culture require infinitely more patience and time, and training and experience.

RADIATION ENERGY AND HUMAN POPULATION

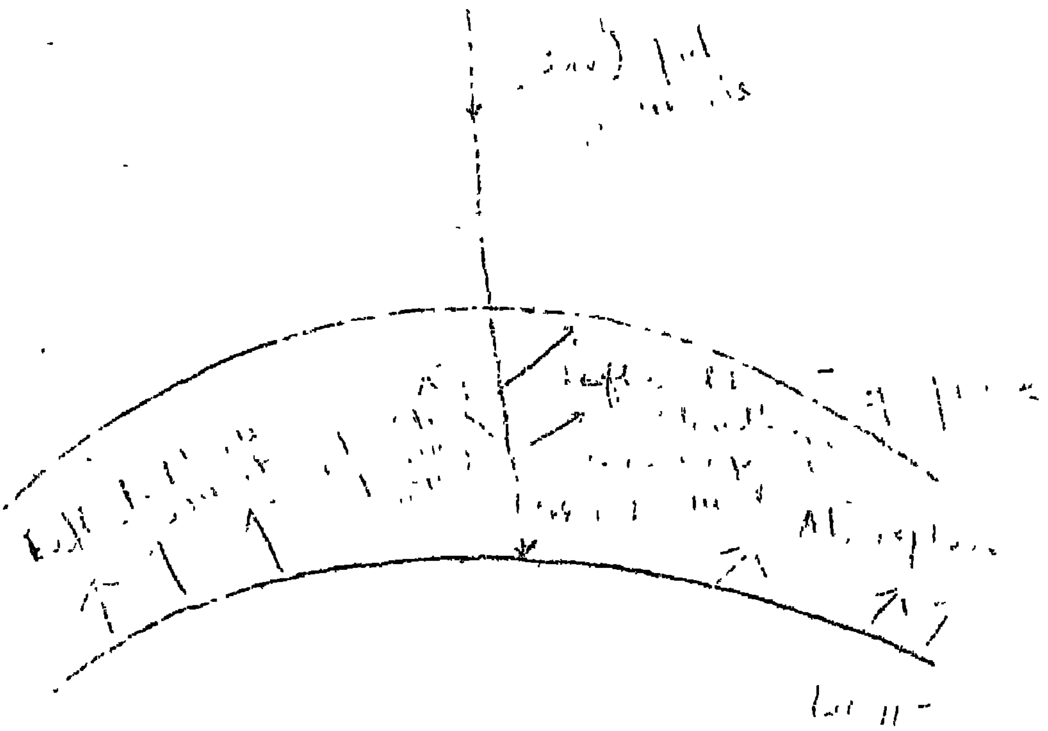
Our planet - Earth- an insignificant speck in the endless ocean of universe - is unique as being the only planet (at least uptil now and till it is proved otherwise) where life exists. Inspite of the fact that life exists in diverse forms, yet there is an underlying unity. Life, wherever it may exist, is organised on the same principals and all operate in the same way; i.e. energy fixed by plants flows through them; nutrients are deposited inthe tissues of organisms, cycled from one group to another; and finally releazed by decomposition to soil and water for recycling. Unmistakably thus solar energy is the prime mover of the universe and man tries to know more about and understand this phenomenon in order to control, conserve and use it in everyday affairs. Human population like those of other animals and plants, are intricately involved in the energy relations of the whole earth. Apart from radiation energy from the prime source Sun, we harness energy from fossil fuels, wood and charcoal, in water, wind and nuclear reactors.

Different kinds of energy show themselves in both physical and biological systems and the law of conservation of energy is experimentally demonstrable in living systems. Although the energy may be converted from one form into another yet the conversions are equational or quantitatively equal. In other words the energy may be changed from one kind into another, but it can't be either created or destroyed. However, the efficiency in the conversion of energy within the various physical and biological systems is not very high. For example, an internal combustion engine is less than 30% efficient in the conversion of chemical energy (fuel) in to mechanical energy (motion); the remainder is lost largely as heat that escapes from the system into the environment. Although tremendous quantities of solar energy are available to the green plants (producers) yet they are efficient upto the extent of 1-5% only in converting it into chemical energy for photosynthesis. Indeed major portion of the natures gift is lost into the environment as Heat. Just imagine what type of earth we would be living in if our green plants were 100% efficient in converting the solar energy into chemical energy with the help of Chlorophyll and produce carbohydrates for ever increasing human population of the globe ! Inspite of the low efficiency the utilisation of solar radiation is by far the greatest source of energy inputs and accounts for 99.98% of the total as such it is imperative to understand its nature and composition. Solar Radiation from Sun comes to us in the form electromagnetic waves with wavelengths ranging from very short waves like (X-rays, r rays, U.V.rays and cosmic rays) to very long radio waves but about 99% of the total energy present occurs in the region of the wave lengths 0.2 to 4.0 microns (i.e. U.V. to inpra-red and about 50% of this energy occurs within the wave lengths between 0.39 to 0.76 microns i.e. visible spectrum.

The above facts suggest that solar radiation is principal ultimate source of light, heat and chemical energy on which human populations depend. It has already been pointed out that a very small percentage of solar radiation striking the earth is captured by green plants, converted to chemical energy and stored as food. It is this minute fraction of the solar radiation in which not only man but all living things depend for the continuation of their life processes as the ultimate source of chemical (food) energy. The process whereby green plants fix the energy of the sun is called photosynthesis. Photosynthesis - a complex process completed in several complicated chemical reactions - is controlled by many structural, physiological and environmental factors with regard to its efficiency in nature. Some of these are the temperature availability of minerals and water, the intensity and duration of Sunshine and the fine structure of chloroplast. It is this photosynthetic efficiency which is of direct concern to man. In short photosynthetic efficiency or Assimilation efficiency is expressed by

$$\frac{\text{Energy captured by green plant}}{\text{Total solar Radiation available}} \times 100$$

In order to understand the above expression, we should have some idea regarding the Solar Input. The Solar energy per unit area falling on earth is called the Solar flux which has been estimated as 2 Cal/minute/Cm^2 or a total of 1.3×10^{23} Cal/Yr. Fate of this Solar input as it passes through the atmosphere is shown diagrammatically as follows.



As is seen from the above figure only 48% of the total Solar input, actually reaches to the surface of the earth and at places 80% of this is reflected back to the atmosphere by the shining objects like Sand, Water, Foliage etc. In general, only about 25% of the sun light reaching the ground is of wavelengths that stimulate photosynthesis, and only a fraction of this 25% is actually used by green plants to produce the dry matter or Biomass. On the average, throughout the world 320 gm dry weight of the green plant matter is produced per square meter per year. Assuming that 4 Kcal of Solar energy are incorporated to produce 1 gm dry plant matter as result of photosynthesis, the total energy incorporated by the green plant amounts to 1.28 X 10^6 Cal per square meter per year. Here we see that photosynthesis is expressed in terms of unit area and unit time. This expression of Photosynthesis is termed as the primary productivity. Thus 320g dry weight/m^2/Yr or 1.6 X 10^{16}g dry wt/year/ for whole earth or 4.8 X 10^{20}Cal/per year/For whole earth or 1.28 X 10^6 C_al/Sq meter/Year are different ways in which the Pr. productivity due to green plants is expressed. To explain the concept of Primary productivity Let us start with 10000 corn plants growing in an acre of land for one year. The harvested material at the end of one year (Say 600 Kg dry weight) represents the standing crop or Biomass expressed as 600 Kg dry Wt/acre. But it should be remembered that this Biomass has accumulated through a period of one year's growth as such the primary productivity is expressed as 600 kg dry Wt/acre/year or 1.6 kg dry Wt/acre/day. The values given above don't represent the actual amount of photosynthesis because during this growing period of 1 year the corn plants have used substantial amount of photosynthetic products for their metabolism through a process of Respiration. Thus the above expression represents only the total photosynthesis minus total respiration per acre per year. This type of primary productivity is known as Net Primary Productivity (NPP). If to this NPP the values of total respiration are added we get an expression for total productivity or Gross Primary Production (GPP). In other words

GPP = NPP + R

or NPP = GPP - R .

(As a Corollary - GPP may be looked at as Gross salary of a person, Respiration as the total expenses and NPP as the saving that can be kept and used at some other time).

As is the usual case man's interest always lies in the maximum savings and reduction in the expenditure. Similarly, man as an agriculturist is more concerned about the NPP and tries various means to reduce the respiration of his green plants but inspite of his best efforts; overall efficiency of land surfaces as a whole are around 0.1 to 0.3% with estimates in water under natural conditions being fairly similar. As indicated earlier it is possible to estimate the efficiency of NPP calculation on input of solar energy during the growing season. Based on 500 calories/Sq cm/day of energy input, potential net plant production (GPP-R) is about 71 g/cm^2/day. This converts to 26.6 calories/cm^2/day of solar radiation which results in biomass or 5.3%of the total incident solar radiation and 12% of the energy received as visible light (222 Cal/cm^2/day).

The following tables gives you a rough idea about the comparison between the productivity of natural plant communities and agricultural crops developed for high productivity.

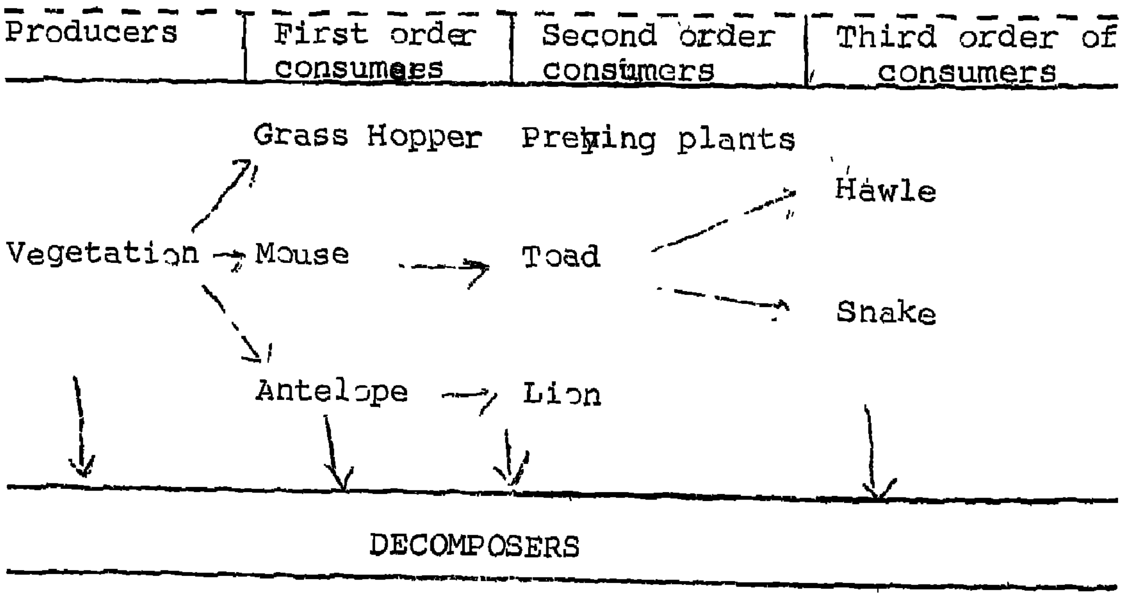
Nature of community	WPP(I/m^2/day)
Natural systems	
Pine forests	6.0
Deciduous forests	3.0
Tall grass prairie	1.22
Short grass prairie	0.19
Deserts	0.11
Fresh water pond	2.6
Sewage ponds	15.3
Marine (Algae)	0.7
See weed	5.4

Nature of community	WPP(I/m^2/day)
Natural systems	
Open Ocean	0.22
Costal zone	0.42
Agriculture systems	
Temperate zone	
Corn (Israel)	9.4
Corn (U.S.A.)	7.2
Corn (Japan)	3.01
Tropical zone	
Sugar cane (Hawai)	19.7
Rice(Srilanka)	0.9

The net productivity (includigg stems, roots, leaves, fruits and grains) of the most productive agriculture is around 6000 to 10,000g/m^2/yr though the net production in most agriculture is around 1000-3000 g/m^2/yr. inthe same general range as many forests. As can be seen from the table some natural ecosystems (communities) are highly productive and others conspicuously unproductive.

From the stand point of energy resources and efficiencies of ecosystems, the first major loss of food resources is in respiration by green plant itself and this is the factor which accounts for the differences between gross productivity and net productivity. The actual energy lost as a result of respiration by the procers of the ecosystems varies widely not only with environmental factors but also with the structure of the ecosystem. For example, young ecosystems and agricultural ecosystems need not to support a large mass of living tissue as must a mature forest. The forest leads to consume a greater proportion of gross productivity than the agricultural crop. Over all, green plant respiration may be considered to account for about 30 - 40% of the G.P.P. so that the N.P.P. is about 60-70% of G.P.P.

On a world-wide basis almost all of the energy fixed and stored by green plants (producers) is consumed by other living organism. Much of that which is not consumed by herbivores and omnivores is consumed by decomposers. Fig.   represents the several nutritional levels from producers to herbivores to primary and secondary carnivores and decomposers - jointly referred to as trophic levels.



It is possible to describe the specific nutritional relationships of a community in terms of the species involved. Such a series of specific relationship is a food chain. In actual communities of living things, there are ordinarily numerous species identified with each trophic level and numerous simple food chains operate simultaneously in a community and become intricately inter connected to form a food web . A knowledge of precise inter-relationships of the food web of a given ecosystem is needed if man is to be aware of the full utilizations of any planned ecosystem modifications. Of more immediate concern here is the energy utilization efficiency at the various trophic levels. The NPP represents the food produced beyond the consumption of food by the producer. If the herbivore feeds on this food, it will convert some of the food materials into living tissue or biomass of its body but will consume much of the food for energy in the process of Respiration. A similar situation exists for the Carnivore, the secondary Carnivore and so on. If we now view these trophic levels as potential food (or energy) sources for man, it is apparent that the efficiency of total energy available as a food source at each successive trophic level decreases. As would be expected, the actual values at each trophic level vary widely from ecosystem to ecosystem and from food chain to food chain within an ecosystem.

Numerous energy factors and relationships operate in all ecosystems. Often there is importation of organic matter from other ecosystems and there may be indefinite storage of organic matter from any of the trophic levels and some is exported. Much of the energy efficiency loss from a lower trophic level to a higher one is due in part to energy consumed in respiration. Some of the energy remains unavailable to the organisms of the next trophic level because it is stored and can be exported to another ecosystem or used by bacteria and fungi in the process of decay

Since man is also dependent upon the food energy incorporated from the incident solar radiation, the comparative efficiencies of various food sources are of human interest. This is particularly true in relation to current human population growth and the existing inadequate diets of many such population. It is estimated that the average daily per capita consumption of energy from food is about 2200 K.Cal averaged over the entire world. Man's daily energy requirement is about 1,320 K.Cal/day when at rest and 2,400 K.Cal if moderately active. Assuming the average basic needs of man as 2,500 K.Cal/day if this entire energy is derived from herbivores the requirement would be equivalent to about 25,000 K.Cal of herbivores which would in turn require about 250,000 K.Cal/day of the producer. Of the producer is about 1% efficient, this would in turn require about 25 million K.Cal/day of radiant energy. Based on solar radiation of 500 $Cal/Cm^2/$ day, it wold require between 0.4 and 0.6 hectares to feed one person for one day. The 1.4 X 10^9 hectares under cultivation and 2 X 10^9 hectares of grazing land on earth provide the 3.5 X 10^9 people on earth with about 0.4 hectares per capita under cultivation and 0.6 hectares of grazing land. The total potential arable land is about 3.2 X 10^9 hectares and total grazing land is about 5.2 X 10^9 hectares. While it is possible to subsist on around 2,000 K.Cal/day, it is not possible to be very energetic or function effectively in industrialized society at that level of energy intake. The implication is that under the above assumptions and estimates of efficiencies it would not be possible to feed more than about 2.5 times the present world population. In actual fac . the diet of the world human population is made up of food taken in at various trophic levels with a wide range of cultural and individual differences due to ecological, social, religious and personal preferences.. But the advantage of using the producer level is obvious.

It is to be noted that this final comment is distinctive to man as highly adapted, socialized animal.

The account given on human food needs clearly implies the importance of how land should be used, in order to obtain the maximal efficiency from the soil, the vegetation (both natural and man-made) and from natural faunas and domesticated livestock that feed upon the particular land areas. Out of about

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3,000 million metric tons of food that is produced annually, about 2% comes from water in the form of fish. Food from land comes mainly from agricultural land which is only 10% of total land area. The rest comes from land 51% of the cultivated land produces cereals, 1.6% potatoes, 4.5 pulses and 8.6% oil seeds. These plus sugar constitute 66% of the total food by weight. Vegetables, fruits and nuts constitute another 16% of the food and these also come mainly from arable land. The remaining 18% is contributed by meat, milk and egg. Fig. shows comparative land areas, population and food production of world, Asia, China and India.

Asia possesses a little over 31% of worlds cultivated land but has more than 55% of the worlds population. China and India, the two most populated countries of the world, together possess 19% of the cultivated land but about 37% of the population. Though India has more agricultural land than China, production of plant food is considerably less.

As already noted in modern agricultural societies, about 0,4 hec. of fertile land is required to feed one person on continuous basis. The following table will give you an idea about the present status of different region of the world with regard to availability of arable land.

Countries	Available land/capita
Denmark France & Sweden	0.4 ha - Self sufficient
India & Pakistan	0.3 ha - Capable of self sufficient & at times import food.
China U.K.	0.2 ha 0.16 ha - It must import food.
Srilanka, Korea	0.2 ha
U.S.	1,16 ha - Export large
Australia	1.6 ha - quantities of
Canada	2.6 ha - food.

Clearly, as population density increases the land per capita decreases. Under these condition productivity must increase if per capita food supply is to remain at the same level. From the above it follows that provision of adequate diets throughout the world will be very difficult unless population growth is slowed. In the meantime, it is imperative that every effort be made to increase food production and availability. Most methods for increasing food availability depend upon producing more food and the many procedures for increasing food production are the use of fertilizers, pesticides and irrigation. Coupled with efficient techniques for cultivation, harfestation, and storage; and finally through selective breeding productivity can be maximised.

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Concepts of Environmental Pollution

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Pollution:

Used in its broad sense, the term "Pollution" refers to any change in the natural quality of the environment brought about by chemical, physical or biological factors.

In 1965, U.S. President's Science Advisory Committee, Environmental Pollution Panel, restoring the quality of the Environment gave "Pollution is the unfvourable alteration of our surrounds wholly or largely as a by-products of man's action through direct or indirect effects of changes in energy patterns, radiation levels, chemical and physical constitution and abundance of organisms. These changes may affect man directly or through his supplies of water and of agricultural and other biological products, his physical objects or possessions or his opportunities for recreation and appreciation of nature.

Pollutants are numerous gases (such as sulphur dioxide and Nitrogen oxides) and particuleta matter (such as smoke particles, lead aerosols, and asbestos), in the atmosphere, pesticides and radioactive isotopes in atmosphere and in water-ways sewage organic chemicals and phosphates in water, solid wastes on land, excessive heating(thermal pollution) of rivers and lakes and many others. Pollution nearly always refers to the consequences of human activity clearly most drugs and food additives together with those industrial poisons which present purely occupational hazards. Some pollutants cause particular concern because they are described as poisons. Even such non-poisonous substances as glucose and common salt can be injurious or lethal when they are present sufficiently higher concentrations in living organisms. By the same token, familiar poisons such as carbon monoxide and arsenious oxide are apparently harmless at relatively low concentrations. In practice, poisonous pollutants in the living environment are usually present at levels well below those of known to have harmful effects.

The affluent societies of the developed nations of the world are likely to be more concerned about the unfavourable effects than those nations in which poverty and hunger are major unsolved problems. The unfavourable effects of pesticides and fertilisers run off are likely to be of less concern in a country in which insufficient food production is leading to malnourishment and starvation or in which insect-born diseases are major contributors to human morfidity and mortality than in a country which agricultural surpluses relatively pure water supplies and a strong public health programme.

Poverty, starvation and pollution all reflect mankind's failure to design social and political institutions capable of properly assessing and controlling technological innovations. Serious problems of poverty and hunger exist in the United States despite the progress of the last several decades and the progress that has been made has been accompanied by the aggravation of many existing environmental problems and the production of new ones.

Levels and movement of pollutants.
Natural and man-generated pollution.
Almost completely man-generated.
Chlorinated hydracarbons(DDT etc.) Lead aerosols.
Substantially man-gerated.
Oil in the ocean.
Phosphates in running waters.
Substantial contributions from natural sources
Hydracarbons in the atmosphere
Radiation
Sulphur oxides in the atmosphere.

Concentrations of Pollutants:

Concentrations of pollutants are often emphasised by fractions.

1 ppm corresponds to one part pollutant per one million parts of the gas, liquid or solid mixture in which the pollutant occurs.

In the case of gas mixtures, the reference is generally to ppm by volume, whereas in the case of liquids and solids the reference is generally to ppm by weight. In recent years it has become customary to express gaseous pollutant and particulate matter in the atmosphere in mass density units of micrograms per cubic metre.

Fractional Concentrations:

Symbol	Definition	Fraction
ppm	Parts per million	10^{-6}
pphm	Parts per hundred million	10^{-8}
ppb	Parts per billion	10^{-9}
ppt	Parts per trillion	10^{-12}

At first glance, a concentration of 1 ppm seems radiculously a smaller and negligible. Nevertheless concentration of pollutants at levels of 1 ppm or less can have serious adverse effect.

1 ppm of phenol in water is lethal to some species of fish.

0.2 ppm average SO_2 level in the atmosphere has been shown to lead to an increased human mortality rate.

0.02 ppm peroxybenzyl nitrate is smog can cause severe eye irritation in humans.

Long distance movement of pollutants

There is accumulating evidence that many types of pollutants can become distributed over the whole earth in relatively sharp period of time.

Radio Active fall out: Through out the world from atmospheric nuclear tests within few days.

World wide distribution of DDT and its metabolites. These were first developed for pesticidal use in the late 1930 and released for civilian use in 1945; since this time they have been widely used throughout the world, they are now widely distributed throughout the marine ecosystems of the pacific ocean, for example probably having been transported by air.

Levels of DDT and its metabolites of over 100 ppb have been found in the liver and fat of animals that never ~~leadxtaxx~~ leave the Antartic ice peck, such as Adelie penguins.

Persistence:

Some pollutants remain dagerous indefinitely Beryllium and Lead being examples. Others that are eventually broken down into harmless compounds may still persist in the environment for long periods of time. The time needed for the pesticide level to be reduced to less than 25% of the original level applied, estimated

5 years for chlorodane
4 years for DDT
3 years for ~~Dexxxxxx~~ Dieldrin
1.5 years for Picloram
5 months for 2,4,5-T and
1 month for 2,4-D

It has been estimated that inorganic mercury compounds in bottom sediments of lakes and rivers may take 10 to 100 years to be converted into methyl mercury, an organic form a dangerous to animal life. Radioactive nuclei decay exponentially with a wide range of half-life, the half-life being the time necessary half of the original number of nuclei to decay.

Isotape	Half-life
Be^{7}	53 days
Kr^{85}	10.7 years
Sr^{90}	28.9 years
I^{131}	8.1 days
Cs^{137}	30.2 years
Ra^{226}	~~10~~ 1,602 years
Pu^{239}	24,400 years

Biological concentration

Another important characteristics of pollutants is that they may be concentrated biologically so that levels in one part of an ecosystem are much larger than those in other parts.

This typically occurs in food chains: levels in an organism are higher than those in the food. Hickey et al, 1966 studied lake Michigan ecosystem found DDT levels of

0.014 ppm (wet weight) in mud sediments on the bottom.

0.41 ppm in bottom feeding crustacea.

3 to 6 ppm in various fish (alewives, chub

Over 2400 ppm in the body fat of fish eating gulls.

One reason for this sort of concentration is that chlorinated hydrocarbons are much more soluble in fat than in water.

Through the food chain is the exceptionally high level of Cs^{137} found in human living far north of Europe, North America, particularly Lapps and Eskimos.

In this instance, Cs^{137} from fallout was concentrated in Lichens, then in Reindeer and Caribou feeding on the lichens, and finally in human eating those animals, body budens of Cs^{137} were 10 to 100 time greater than those occurring in human at more temperate latitudes.

Synergism and Antigonism: It is impossible to discuss the effect of pollution probably nearly be discussing the effect of individual pollution. In many cases, the combined affect of two or more pollutants are more severe or even qualitatively different from the individual affects of the separate pollutants - a phenomenon known as Synergism.

Wide spread injury to Peanut crops in Texas in 1966 and 1967 were identified due to synergistic action of oozone and SO_2.

Numerous studies have shown that some types of particulate matter, such as aerosols of soluble salts of ferrous ions, manganese and venadium can increase the toxicity of SO_2. Such an increase in toxocity usually referred to as potentiation.

Sometimes the combined effects of two pollutants are less rather than more severe and this refers to as antagonism.

Eg. Cyanides in industrial wastes are quite poisonous to aquatic life, and in the presence of zinc and cardmium, they are extremely poisonous (a synergistic effects), apparanel due to the formation of complexes; in the presence of nickel, however, a nickel cyanide complex i.e. not very toxic is formed. (an antagonistic effect) The occurrence of synergistic effects makes it difficult to study the effect of pollution since so many pollitants are present in the environment.

MENDEL'S PRINCIPLES OF HEREDITY AND NON-MENDELIAN INHERITANCE

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Mendel's Laws of Heredity

Gregor Mendel (1822-1884) is appropriately called as the"Father of Genetics". He made revolutionary contributions with his experiments on garden pea, Pisum sativum, conducted in a monastry garden and thus laid the foundation for the Science of Genetics. Mendel was a modest man who graduated from High School in Brun (Austria) (Now Brno in Czeckoslovakia). He entered in Augustinian monastry and was ordained priesthood in 1848. He has a great desire to become teacher. He left Brunn and studied at the University of Vienna for two years. When he returned from Vienna he again became teacher in Brunn's Modern School where he served for 14 years. He then joined the Church and started his experiments on garden Pea in 1856 in small garden of the monastry. After 9 years in 1865 he published his historic paper in the "Proceedings of the Brunn National Science Society". No one realised the importance of his contribution and his work remained unnoticed for 35 years. In 1900, Mendel's paper was discovered simultaneously by three botanists: Hugo de Vries in Holland, Carl Correns in Germany and Ercivon Tschermak in Austria. It was only then that his work was recognised much after his death in 1884.

Mendel was lucky to have garden pea as a plant for his hybridization experiments. In this plant, there are several varieties which exhibit qualitatively different characters. He could recognise seven such contrasting expressions of characters. These characters as investigated by Mendel are as below:

Charavter	Contrasting Expression
1. Form of Seed	Smooth : Wrinkled
2. Colour of Cotyledon	Yellow : Green
3. Colour of seed coat	Grey-brown: White
4. Form of pods	Inflated : Constricted
5. Colour of unripe pods	Green : Yellow
6. Position of flowers	Axial : Terminal
7. Length of stem	Tall : Dwarf (6 to 7 ft) (9 to 18 inches)

By selecting the above seven pairs of contrasting characters Mendel was lucky that these characters neither showed any linkage, epistatic reaction or quantitative inheritance. With his background of mathematics, Mendel was a better Statistician than a naturalist. And with his great perseverence and dilligence he could deduce his laws of inheritance from his hybridization experiments on garden pea. Pea plants are very favourable for hybridization. Self pollination in then can be easily prevented by carefully removing the anthers before they open in the bud, and cross pollination ~~at the~~ easily effected by smearing the stigma at the receptive stage with the required pollen.

Monohybrid Cross

Mendel started his experiments by taking into consideration these seven pairs of characters, one character at a time. Such cross are called as monohybrid cross. In one such experiment, Mendel raised two varieties of garden peas, tall and dwarf. When self-fertilised, tall plants always gave rise to tall while dwarf plants gave rise to dwarf plants. Mendel then crossed these two varieties. From this cross, all of the offspring in the first generation were tall - The dwarf plants have disappeared from the F_1 generations.

Prior to Mendel, the general belief ~~that~~ was that dissimilar characters, when crossed, would blend and result in intermediate character in the offspring. Mendel, however, found that there is no blending of characters in the ~~offsp~~

offspring, the character of only one present was seen in the offspring, the other character seems to have disappeared.

Mendel did not stop here. He self-fertilised the plants of the F_1 generation. In the second generation, Mendel found that the character which has disappeared in the first generation reappeared in some plants of the second generation. Careful classification of the plants in the second generation showed that about 3/4 of the total plants were tall, while 1/4th were dwarf. Such 3:1 ratio always appeared in all the monohybrid crosses. Mendel designated the character that appeared in F_1 as *dominant* and the character that reappeared in a smaller number of plants in the second generation as "*recessive*". Thus in F_2 generation, about 75% of the plants showed the dominant character and about 25% of the plants showed the recessive character. With these results Mendel speculated and arrived at the following assumptions to explain his results.

1) There are definite hereditary units(Mendel called them *factors*; we now call them *genes*) which are responsible for the transmission of characteristics.

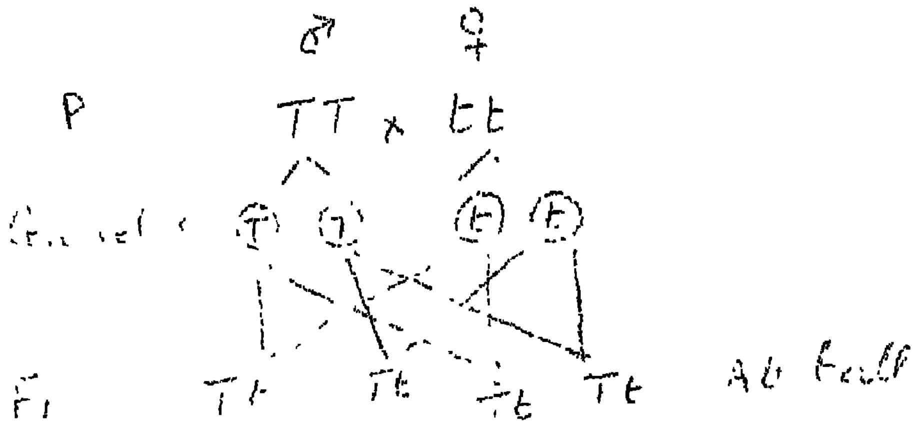
2) Each character in an organism results from the presence of the "factors", one derived from each parent. This is also called as *Law of Unit Character*.

3) The two genes responsible for a character may be similar or dissimilar. If dissimilar, one of them will be dominant and the other recessive. If the dominant and recessive are present together, the dominant character alone will be expressed in the body of the organism and the recessive will remain marked by its dominant counterpart. The two genes that represent one character are now called as *alleles*. This is also called as *Law of Dominance*.

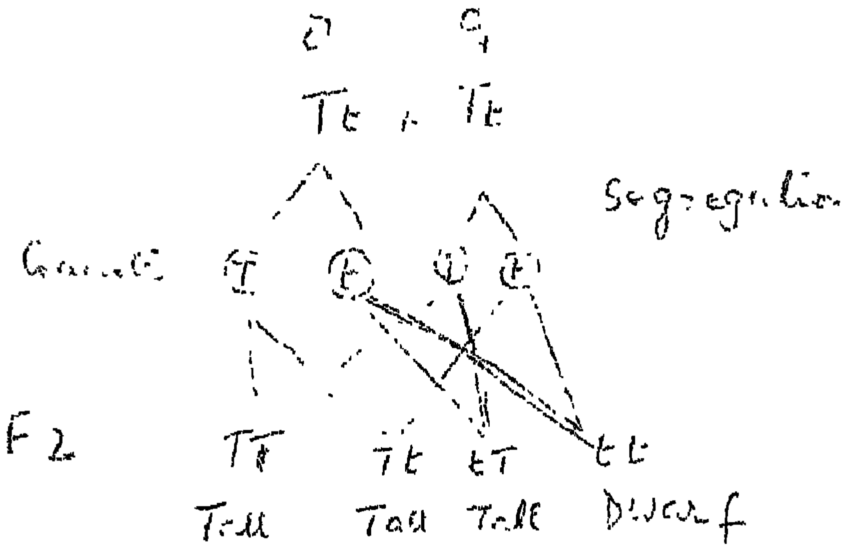
4) *Law of Seggregation*: The two alleles that remain together in the body of an organism separate and go to different gamets at the time ~~of~~ the organism produces gamets for the purpose of sexual reproduction. So a gamete will have only one allele of a character (purity of gamets), and never both the alleles.

5) The separated alleles are again brought together by the union of two gametes during fertilisation. The gametes units at random without any regard to the alleles contained in them.

Mendel devised the system of using letters as symbols for the factors (the heredity determiners). He used small letter for a recessive factor and a capital letter of the same kind for the dominant factor. Thus, he used capital "T" for tallness and small "t" for dwarfness Let us now represent the mnohybrid cross by way of using symbols and see how Mendel put the result of these monohybrid (one character) crosses.



All the progeny of the first generation were tall plants, after following the law of dominance. Mendel went further, and self-fertilised the plants of the first generation, as shown below:



Again following the law of dominance, Mendel found that 3/4th of the plants in the F_2 generation are tall(TT & Tt), while 1/4th of the plants are dwarf (tt).

At this point we must introduce two other genetic terms which will make our discussion much easier. The tall plants are of two kinds according to the genes which they carry (TT and Tt), but only of one kind according to the expression of the genes (Tall). When we refer to the type of genes we use the term Genotype and when we refer to the expression of the genes we use the word Phenotype. Thus in the above cross the phenotype ratio is 3:1 i.e. three tall (TT,TE,ET) and one dwarf (EE), while the genotype ratio is 1:2:1 i.e. TT: TE or ET : tt.

Genotype: Genetic constitution of an organism.

Phenotype: The physical appearance of an organism resulting from its genetic constitution or genotype.

Mendel studied all the seven pairs of alternative characteristics and all behaved and showed the similar results as were shown by tall and dwarf plants. The following table shows the results as Mendel found in various different monohybrid crosses of the seven pairs of contrasting characters.

Results of Mendel's First Experiments on Seven pairs of characteristics in garden pea

Characteristics	F_2 Results		Ratio
1. Form of Seed	5474 Smooth	1850 Wrinkled	2.96:1
2. Colour of Cotyledon	6022 Yellow	2001 Green	3.01:1
3. Colour of Seed Coat	705 Gray-Brown	224 White	3.15:1
4. Form of Pods	882 Inflated	299 Constricted	2.95:1
5. Colour of Unripe Pods	428 Green	152 Yellow	2.82:1
6. Position of flowers	651 Axial	207 Terminal	3.14:1
7. Length of Stem	787 Long	277 Short	2.84:1
8. All Characteristics combined.	14889 Dominant	5010 Recessive	2.98:1

We may also introduce two more terms here, for our better understanding.

Homozygous: When an organism is having identical alleles at two corresponding loci (TT, or tt).

Heterozygous: When a organism is having two different alleles of a single gene (TE).

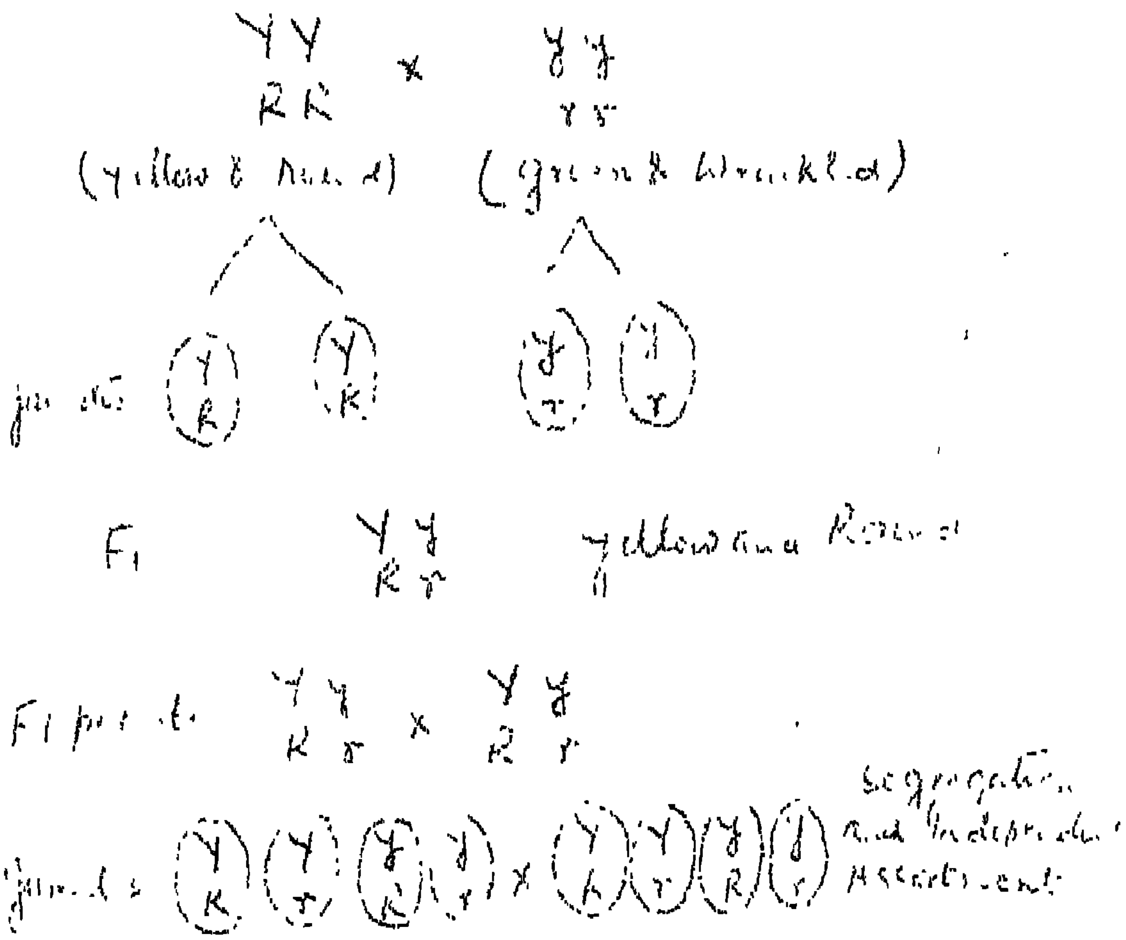
Dihybrid Cross:

After establishing the behaviour of monohybrid cross, Mendel directed his attention to dihybrid crosses, that is, crosses which involve two different characters that are separately inherited. One of these crosses involved the Colour and Shape of the seeds after drying. He found that some of plants produced round (smooth) seeds while others gave wrinkled seeds. A monohybrid cross has already shown that round was dominant over wrinkled. Again some seeds turned yellow when dried while others remained green, and in this case yellow is dominant over green. When he crossed plants producing yellow-round seeds with others producing green wrinkled seeds, he obtained all yellow-round seeds in the F_1 generation. He than planted these seeds and self-fertilised these plants to find out the results in the F_2 generation, and found four distinct types of seeds were produced. in the ratio of 9:3:3:1, giving rise to four different phenotype as below:

9: Yellow and round seeds
3: Yellow and wrinkled seeds
3: Green and round seeds
1: Green and wrinkled

In all he collected 556 seeds out of which 315 were yellow and round, 101 yellow and wrinkled, 108 green and round, and 32 green and wrinkled, roughly in the ratio of 9:3:3;1 as mentioned above. Out of these sixteen 9/16 showed both dominant characters, 3/16 showed one dominant and one recessive, 3/16 showed other dominant and other recessive and 1/16 showed both recessive character. Thus Mendel obtained not only the two parental combinations, but also two new combination resulting from other mixtures of the characters than those found in the two parents. This showed that two factors do not tend to stay together in the same combination in which they are found in the original parts P_1. This separateness of behaviour among genes is called as the Principle of Independent Assortment. According to this principle, the separation or segregation of allelic genes of gene pairs on one pair of homologous chromosomes during meiosis is entirely independent of the segregation of allelic genes on other pairs of homologous chromosomes.

Using the appropriate symbols, Mendel's dihybrid ratio can be represented graphically, as below:



Each of the F_1 parent gives rise to foun types of gametes. when put on the Punnet square we will get the following results

	RY	Ry	rY	ry
RY	RYRY	RyRY	rYRY	ryRY
Ry	RYRy	RyRy	rYRy	ryRy
rY	RYrY	RyrY	rYrY	ryrY
ry	RYry	Ryry	rYry	ryry

Phenotypeic Ratio will be : 9:3:3:1

Gento typic Ratio will be : 1:2:2:4:1:2:1:2:1

This is explained in the following charts

Geno Type	No. of individuals	Pheno type	Total No.of each percenta
1. RRYY	1	Round Yellow	9
2. RRYy	2	,,	
3. RrYY	2	,,	
4. RrYy	4	,,	
5. RRyy	1	Round Green	3
6. Rryy	2	,,	
7. rrYY	1	Wrinkled Yellow	3
8. rrYy	2	,,	
9. rryy	1	Wrinkled green	1

Out of the above, the genotype RRYY and rryy will breed true on self-fertilisation, because they are homozygous for both the characters and resemble the original parents. All the other fourteen are heterozygous for one or both the characters and will segregate in different ratios in the next generation.

Non-Mendelian Inheritance:

Not that all the hereditory crosses always show Mendelian ratio. Infect most of the inheritance shows non-Mendelian ratios, due to a variety of other factors. Some of these factors include, intermediate dominance, semi-dominance, Co-dominance, epistatis and gene interaction. We shall now discuss some of these modified Mendelian or non-Mendelian crosses and the ratios obtained therein.

1) Intermediate Genes

So far we have discussed that one of the allelic gene pair behaves as dominant while other is recessive. There are some cases, however, in which the two genes seem to be of about equal strength in their determining powers and a heterozygous individual shows characteristics which are somewhat in between the two unmixed types. For example if one crosses red flowered four-o'-clock, *Mirabilis jalpa* with white flowered variety, the resulting plants produce neither red nor white flowers, but pink ones, - intermediate between the red and white. If two of these pink-flowered plants are crossed, they produce red, pink and white flowers in the ratio of 1:2:1. Out of these four, one will be homozygous for red, one for white and two will be heterozygous and will be pink. From these results it is evident that wherever a plant carries both the genes for red and white, it will be pink. In other words, neither the gene for red or white is dominant or recessive. This is the case where both genes express themselves partially. Such genes are called as Intermediate. We must remember here that there is no mixing or blending of genes for red and white. They again get segregated in the next generation and express their red and white character. These genes are also called as Semi-dominant genes and are usually represented by the Symbol I.

I^R - Red
I^W - White

P $I^R I^R$ x $I^W I^W$
(Red) (White)

Gametes: (I^R) (I^R) (I^W) (I^W)

F_1 $I^R I^W$ Pink

$I^R I^W$ x $I^R I^W$
(I^R) (I^W) (I^R) (I^W)

F_2 $I^R I^R$ $I^R I^W$ $I^W I^R$ $I^W I^W$
Red Pink Pink White

The phenotypic ratio in their monohybrid cross will be 1:2:1 i.e one red, two pink and one white flowered plants. The genotypic ratio will also be same i.e. 1:2:1 (I^RI^R, I^RI^W, I^WI^W).

Let us now see the dihybrid ratio when both the pairs of genes are intermediate in nature. In the snapdragon *Antirrhinum majus* for example, there are plants with board leaves, narrow leaves and the heterozyous condition of the two will produce intermediate size leaves. Similarly there are plants with red flowers and white flowers and the heterozygous condition gives pink flowers. We now select two plant varieties, each homozygous for each of the two characters, as shown below.

$\begin{matrix}BB\\RR\end{matrix}$ - Plant with broad leaves and red flowers (homozygous for broad and red)

$\begin{matrix}B'B'\\R'R'\end{matrix}$ - Plant with narrow leaves and white flowers (homozygous for narrow and white)

P $\quad \begin{matrix}BB\\RR\end{matrix} \times \begin{matrix}B'B'\\R'R'\end{matrix}$

Gametes $\quad \begin{matrix}B\\R\end{matrix} \quad \begin{matrix}B\\R\end{matrix} \quad \begin{matrix}B'\\R'\end{matrix} \quad \begin{matrix}B'\\R'\end{matrix}$

$F_1 \quad \begin{matrix}BB'\\RR'\end{matrix}$

These plants in F_1 generation will be with intermediate leaves and pink flowers.

F_1 parents $\quad \begin{matrix}BB'\\RR'\end{matrix} \times \begin{matrix}BB'\\RR'\end{matrix}$

Gametes $\quad \begin{matrix}B\\R\end{matrix} \quad \begin{matrix}B\\R'\end{matrix} \quad \begin{matrix}B'\\R\end{matrix} \quad \begin{matrix}B'\\R'\end{matrix}$

	BR	BR'	B'R'	B'R'
BR	BRBR	BRBR'	BRB'R	BRB'R'
BR'	BRBR'	BR'BR'	BR'B'R	BR'B'R'
B'R	BRB'R	B'RBR'	B'RB'R	B'RB'R'
B'R'	BRB'R'	B'R'BR'	B'RB'R'	B'R'B'R'

6

The genotypic and phenotypic ratio of this cross will be 1:2:1:2:4:2:1:2:1 as shown in the table below.

Phenotypes	Genotypes	Genotypic frequency	Phenotypic ratio
Broad Red	BBRR	1	1
Broad pink	BBRR'	2	2
Broad white	BBR'R'	1	1
Int. red	BB'RR	2	2
Int. pink	BB'RR'	4	4
Int. white	BB'R'R'	2	2
Narrow	B'B'RR	1	1
Narrow pink	B'B'RR'	2	2
Narrow white	B'B'R'R'	1	1

In a dihybrid ratio, if only one of the genes behaves as intermediate the ratio received will be 6:3:3:2:1:1. The example of this is taken in case of cattle. The coat colour is either red or white and the intermediate colour is roan. The ~~coat~~ coat colour genes behave as intermediate genes. ~~White~~ While the other character taken into consideration is the presence or absence of horns. The polled (hornless) condition is due to a gene which is dominant over the gene for horned. When polled-red cattle are crossed with horned-white the offspring are all polled-roam. When two such parents are crossed in F_1, we get the following result in F_2 generation with the ratio of x 6:3:3:2:1:1

I^RI^R - Red
I^WI^W - White
I^RI^W - Roam

P = Polled
p = horned

Red Polled X White horned

I^RI^R X I^WI^W
P P   p p

I^R P I^R P I^W p I^W p

F_1 I^R I^W - Polled-roam
P p

I^R I^W X I^R I^W
P p   P p

	$I^R p$	$I^R p$	$I^W p$	$I^W p$
$I^R P$	$I^R I^R$ P p	$I^R I^R$ P p	$I^R I^W$ P p	$I^R I^W$ P p
$I^R p$	$I^R I^R$ p p	$I^R I^R$ p p	$I^W I^R$ p p	$I^W I^R$ p p
$I^W P$	$I^R I^W$ [illegible]	$I^R I^W$ p p	$I^W I^W$ P p	$I^W I^W$ P p
$I^W p$	$I^R I^W$ p p	$I^R I^W$ p p	$I^W I^W$ P p	$I^W I^W$ p p

The genotypeic and phenotypic ratio will be:

6 :	Polled roan	6:3:3:2:1:1
3:	Polled Red	
3:	Polled White	
2:	Horned roan	
1:	Horned Red	
1:	Horned white	

Epistasis:

Epistasis is a widespread type of gene interaction and the term was introduced by Bateson(1907). Any gene or gene pair that marks the expression of another nonallebic gene is epistatic to that gene. The gene supressed is said to be hypostatic. Episatic genes are inactive genes that produce defective enzymes or no enzymes at all. They block particular reactions and mark the effects of normal or hypostatic genes.

Recessive epistasis:

Let us take the example of coat ~~xlaax~~ colour in mice to illustrate the phenomenon of epistasis. The typical wild mouse has a coat colour which is called agouti(a peculiar shade of gray) while some other mice has black coats. The genes for agouti are dominant over those of black which is a recessive character. Mice, like most other animals also carry a few genes for alebinism , which results in a white coat when the genes are homozygous. Albi gene is not an allelic of genes for agouti ~~abd~~ and black. Rather, when homozygous this gene for albinism presents the formation of pigment of any kind regardless of the genes for agouti or black ~~whixe~~ which the mouse may carry. This is due to the fact that the dominant allele of allbino seems to produce an enzyme which is necessary for the formation of pigment and in the absence of this enzyme no pigment is produced. Now if we cross a homozygous agouti male carrying the two dominant alleles of the gene for albinism BBAA, with an albino female carrying two genes for black bbaa. We will obtain all agouti mice in the F_1. When these are crossed among themselves, we obtain agouti, black and albino in the ratio of 9:3:4. The unusual ratio is because of the fact that the albino gene in homozygous condition, aa, are epistatic to both genes for aquotic or black.

BB x bb
AA   aa

B - Agouti
b - black
A - Colour
a - albino

(Agouti) (albino homozygous black)

(B/A) (B/A)   (b/a) (b/a)

B b   All agouti
A a

(B/A) (b/a)   X   (B/A) (b/a)

	BA	Ba	bA	ba
BA	BA BA	BA Ba	BA ba	BA ba
Ba	BA Ba	Ba Ba	Ba BA	Ba ba
bA	BA bA	Ba bA	BA bA	ba bA
ba	BA ba	Ba ba	ba bA	ba ba

9: Agouti   3: Black   4: Albino

Dominant Epistasis:

It results when only one gene of a pair of allele is necessary to influence the expression of a second pair of alleles. The classical example is shown by white ply mouth Rock and white Leg horn chickens. In the F_1 offspring are all white, while in F_2 there are 13 white and 3 coloured chickens. We can explain this result by assuming that the white Leghornes are homozygous for a dominant gene for colour,C, but they do not show coloured feathers because they are also homozygous for a dominant gene which inhibits the formation of colour pigment, I. The plymouth rocks, on the other hand are white because they are homozygous for the recessive gene, c, which fails to produce colour, but they are also homozygous for the recessive gene i which allow colour to form if there were any genes for colour present. The F_1 offpsring are white because the dominant gene I inhibits the formation of colour. But in F_2, 3 Receive the dominant gene for colour without receiving a gene for colour inhibition.

White Leghorn X White Plymouth Rock

$\begin{matrix}CC\\II\end{matrix} \quad X \quad \begin{matrix}cc\\ii\end{matrix}$

C- for colour
c -no colour
I- inhibit colour
i- allow colou formation.

F_1 $\begin{matrix}C & c\\ I & i\end{matrix}$ - White

$\begin{matrix}C & c\\ I & i\end{matrix}$ X $\begin{matrix}C & c\\ I & i\end{matrix}$

	CI	Ci	cI	ci
CI	CI CI	Ci CI	cI CI	ci CI
Ci	CI Ci	Ci Ci	cI Ci	ci Ci
cI	CI CI	Ci cI	cI cI	ci cI
ci	CI ci	Ci ci	cI ci	ci ci

The phenotypic ratio is 13:3

13: White, 3: Coloured

Duplicate Recessive Epistasis:

Another example of modified dihybrid ratio is taken in human beings, the case of deaf-mutism. This defect produces children which are horn deaf and mute. This condition may result when a person is homozygous for either of two recessive genes, let us call them a and b. A person with the genotypic aaBB will be deaf and mute because the gene a is epistatic to B for normal hearing. Similarly a person with the genotype AAbb would also be deaf and mute because the gene b is epistatic to gene A for normal hearing. This is the case of duplicate recessive epistasis, where either of the two recessive genes may be epistatic to the dominant allele of the other - When two deaf-and mute marry, all of the children of F_1 are with normal hearing and speech. But when two such persons of F_1 marry the result obtained in the F_2 will be 9:7 (9 children with normal hearing while 7 will be deaf and mute) as shown below.

P AA bb X aa BB

(deaf mute) (deaf mute)

F_1 Aa bB with normal hearing and speech

Aa bB X Aa bB

	AB	Ab	aB	ab
AB	AB AB	Ab AB	aB AB	ab AB
Ab	AB Ab	Ab Ab	aB Ab	ab Ab
aB	AB aB	Ab aB	aB aB	ab aB
ab	AB ab	Ab ab	aB ab	ab ab

9:7

9: With normal hearing and speech

7: Deaf and mute

Duplicate Dominant Epistasis

In this case of epistasis we get the ratio of 15:1. The feathers on the shanks of some chickens are inherited in this way. Most chickens have unfeathered shanks which result when they are homozygous for two recessive genes. The presence of a single dominant gene for either of the two pairs, however, is sufficient to produce feathrred shanks.

In otherwords, if we assume that the genotype of the birds without feathers on their shanks is ~~as~~ aabb, then the genotypes of Aabb or aaBb (even with one dominant gene of the either pair) would result in feathered shanks.

Gene Interaction:

Domestic breeds of chicken have different comb shapes. Wyandottes have a characterstic type of comb called "rose", Brahmas have "pea" comb, while Leghorns have "single"comb. The rose comb chickens were crossed with those of pea comb. All the F_1 chickens had different comb, called as "Walnut". When the F_1 chickens were mated among themselves, a familiar 9:3:3:1 ratio was obtained in F_2, but the phenotype representing two of the four classes were different from those expressed in the parents. Out of the 16 in the F_2, 9 were Walnut, 3 were rose, 3 were pea and one was single. Neither single nor Walnut was expressed in the original parental lines. These two phenotypes were explained as the result of gene product interaction. The result based on a total of 16, indicated that two different allelic pairs were involved, one introduced by rose comb and one by the pea - comb parents. A gene for rose and pea would interact and produce Walnut as in F_1

	Wyandotte		Brahma
	R R p p	X	r r P P
	(Rose)		(pea)
F_1	R r p P - Walnut		
	R r p P	X	R r p P

F_2: Of the 16, 9 classes are with two dominant genes(R & P) & are Walnut, one class is with full recessive combinatio in rrpp and is single.

The two $\frac{3}{16}$ (rose and pea) classes were R-pp and rrP respectively, with atleast either of the dominant respective gene. Although the usual 9:3:3:1 ratio was obtained, the result from this cross was unusual in two important respects, 1) the F_1 progeny differed from those of either parent, that is, NONE was rose or pea, but all were Walnut and 2) two phenotypes Walnut and single, not expressed in the original parents appeared in the F_2. Genes R & P were non-allelic but each was dominant over its allele (i.e. R over r and P over p). When R and P were together as in the F_1(RrPp) the two different products interacted to produce Walnut comb. The two nonallelic genes R and P acted independently in different ways, similar to the ways in which codominant alleles act. Nonallelic genes behaving in this way are called as Coepistatic genes.

Another example of this gene interaction resulting in 9:7 ratio was demonstrated by Baterson and Punnet in the sweet pea, Lathyrus odoratus. When two white flowered ~~varieties~~ varieties of this plant were crossed, the F_1 progeny all had coloured flowers. When the F_2 were classified, 9 were purple and 7 were white. The base number of 16 was again recognised as that associated with dihybrid cross, but there were only two classes instead of usual four. The hypothesis formulated by investigators was that two nonallelic gene pairs were necessary to produce the purple colour. It has since been shown that in the presence of C and P genes, anthocyanins (pigments) are formed in the flowers. In the absence of either of the Complimentary genes(CCpp, Ccpp, ccPP, ccpp, ccpp) the flowers are white. Nine of the 16 F_2 progeny carried C-P, but seven lacked one or both. The original white parental varieties were CCpp and ccPP and the cross is shown as below

P CC pp (white) cc PP (white)

F_1 CC pp all coloured

C c p p X C c p P

	CP	Cp	cP	cp
CP	CP CP	Cp CP	cP CP	cp CP
Cp	CP Cp	Cp Cp	cP Cp	cp Cp
cP	CP cP	Cp cP	cP cP	cp cP
cp	CP cp	Cp cp	cP cp	cp cp

Phenotypic Ratio is 9:7

9 - coloured flowers

7 - white flowers

The cross between two varieties of white flowers illustrated again both dominance and epistasis. In both gene pairs one allele is dominant over a recessive allele, C is dominant over c and P over p. In addition each recessive homozygote is epistatic to the effects of other gene. As shown above, homozygous cc prevents the expression of purple colour by P and thus is epistatic to P. Likewise, the homozygous allele pp prevents the expression of colour by C and is epistatic to C. Both C and P are hypostatic, C to pp and P to cc.

MODERN GENE CONCEPT

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Our understanding about the nature and meaning of the gene has changed from time to time in keeping with the discovery of new knowledge in genetics. The term 'gene' was first used by Johansen in 1909 to mean some character of an organism. At that time and for several years hence genes were believed to be located as beads on a string, and since the electron microscopes were not developed, the genes remained mysterious or abstract units that could not be seen.

The beads-on-a-string hypothesis received a severe blow from the work on intragenic recombination. This work showed that genes are like the polypeptides they determine, linear structures. Roper and Pritchard demonstrated that crossing over could occur even within a gene; earlier crossing over was known only between different genes.

A decade earlier, Beadle and Tatum (1941) had provided the first convincing evidence about the function of genes, namely protein synthesis. They suggested that one gene determines one protein. A few years later this hypothesis was modified to one gene: one polypeptide. What genes actually determine are not proteins but polypeptides. The polypeptide can be a part of any protein, not just an enzymic protein. If a protein is made up of only a single polypeptide, then only one gene will suffice to make that protein. But if the protein is made of say 3 or 4 polypeptide chains, then 3 or 4 genes will be needed.

It was established from the work of Avery, MacLeod and McCarty (1944) that DNA was the genetic material. The nucleotide sequence of DNA precisely determines the amino acid sequence of polypeptides; from this the nature of the gene could be readily grasped. However, the work of Benzer (1955) on the fine structure analysis of some genes introduced very significant conceptual changes in our understanding of what a gene is.

Contd...P/2.

Benzer stated that, strictly speaking, genes did not exist at all ! He showed that the concept of a gene being a definite structure which determined a polypeptide, mutated, and recombined by crossing over, was erroneus in the sense that these properties did not describe the same thing . While a cistron is a long stretch of DNA containing over a thousand base pairs, a mutation could occur in any one base pair, and intragenic recombination demonstrated that crossing-over involves some intermediate length of the molecule (between 1 to over 1000 base pairs). The terms muton and recon were proposed to define a gene in terms of the smallest units of mutation (i.e. a single base pair) and recombination (a few base pairs). A cistron is divisible into mutons and recons.

In common usage the term gene is used to mean a unit of function, i.e., a cistron. As explained above, a cistron is a much larger unit than muton or recon. Therefore a gene in the sense of cistron is something larger than a gene in the sense of muton or recon.

Further, even the correspondence between gene and cistron suffered a major setback in 1977 when Glover and Hogness discovered split genes (see Glover, 1984). Until then the units of function and transcription were believed to be identical and the term cistron could be justifiably applied to both. In split genes, on the other hand, only the exons (Fig. 1.19) (not introns) are the units of function, and these exons constitute only a small part of the transcribed sequence. Even more serious implication of the split genes was that it dealt a death blow to the once popular one gene: one polypeptide hypothesis because in several cases, the transcribed sequence undergoes splicing during post-transcriptional processing to form different polypeptides; i.e. here one gene can produce several different polypeptides !.

The important point to note is that the word gene can mean different lengths of DNA; a muton, a recon, a replicon (unit of replication), a transcription (unit of transcription), and a cistron are all made of DNA segments, but the length of the segment differs in each case, ranging from the smallest (just 1 base pair) to the longest (over thousand base pairs or even longer).

Contd...P/3.

The 3 end of many eucaryotic genes contains the sequence AATAA (Fig.1.19)(or AAUAA in mRNA) that signals an endonuclease enzyme to cut off the strand at a site some 11-30 bases downstream. Thereafter a poly-A tail is added.

One of the most salient features of eucaryotic DNA is that only a small part of it actually codes for proteins. There is a great excess of DNA than required for gene function. Introns can account for up to 90% of a gene. Genes (i.e. introns + exons) account for only about 10% of the genome (Fig. 1.20). Some DNA sequences are repeated again and again. Adjacent genes are separated sequences. These sequences may be transcribed, but if so, they are degraded during post-transcriptional processing. These repeated sequences probably act like skeletal spacers.

Another kind of nucleotide sequences, called palindromes, contain some 300-1200 base pairs and read the same in both directions, like the word MADAM. When RNA is transcribed from such palindromes, it forms hairpin loops as shown below.

CTATAG C - U - A
transcription CUAUAG —— hairpin
GATATC G - U - A loop

GENE REGULATION

Eucaryotic multicellular plants and animals normally develop by repeated mitotic divisions and differentiations from a single cell, the zygote. From this single cell, different kinds of cell, tissues and organs, of the adult organism are developed. Mitotic divisions imply that the descendents of the zygote are genetically identical to the original, with two copies of each gene per diploid cell. Thus it stands to reason that no matter what may be the reasons for cell differences in the organism, they certainly cannot be due to differences in the types or numbers of genes per cell. Thus the question arises as to how do genetically identical cells

Contd...P/4.

become functionally different during development? During the 1950s and 1960s many leading scientists were attempting to seek answer to this puzzle.

We now know that the clue to orderly structural and functional differentiation of multicellular organisms is the fact that their cells do not constantly produce the same proteins all the time. Even unicellular bacteria show temporal changes in protein synthesis. The cells seem to have some kind of regulatory mechanism which turns the genes "on" or "off" at different times or in different conditions. Differentiation or morphogenesis appears to be the result of systematic, methodical, activation and repression of genetic material at different times.

Theoretically, differences between cell types can originate from various means (see Table 7). In many cases, transcriptional control is employed. In this method, the cell only transcribes those genes which it really needs at a particular time. The first case of transcriptional control studied was in cells of E. coli. These cells only produced enzymes to digest lactose when lactose was the only food source available, otherwise not.

The simplest form of metabolic regulation is feedback inhibition in which the end product of a biosynthetic pathway inhibits the activity of the first enzyme in the synthetic sequence (Fig. 1.21). This means that if the end product is not being used or withdrawn from the system, the synthetic pathway is shut down, and the activity level depends on the utilization rate of the end product.

The important characteristics of feedback inhibition are the following:

1. Only the end product or its anologues are effective;
2. it affects only one early enzyme; and
3. it acts at once, regardless of the presence of existing enzymes.

The end product does not inhibit by competing with the normal substrate for attachment to the enzyme. The enzymes

Contd...P/5.

involved in feedback inhibition appear to have two distinct specificities, one related to its substrate, the other to the end-product inhibitor. This kind of configuration is called allosteric. The small end-product molecule is supposed to attach itself to the larger enzyme molecule and to change the enzyme's shape in such a way that it can no longer bind to its normal substrate.

A different types of control mechanism is repression, also triggered by the end product of a synthetic pathway. Unlike feedback inhibition, however, repression leads to shutting down the synthesis of all the enzymes involved in the pathway, not just the first enzyme (Feedback inhibition has no direct effect on enzyme synthesis).

Both feedback inhibition and repression exemplify negative control systems (Fig.), with the synthetic pathway being shut down by the excess of its end product.

The general mode of gene expression in some bacteria is illustrated in Fig. .

In bacterial cells, genes with related functions often tend to cluster together on the chromosome. Thus nine enzymes are needed to synthesize the amino acid histidine, and the genes for those enzymes occur right next to each other in a single big cluster, arranged in the same order in which their enzymes function in the metabolic route of histidine formation. Similar examples were also observed for tryptophan (5 genes/enzymes), galactose (3) and lactose metabolism (3). Considerable work has been done on lactose metabolism and is briefly explained below.

It seems logical that E. coli can metabolize the sugar lactose since these bacteria live in the intestines of mammals that occasionally drink milk. Since the milk sugar lactose is not normally present in the intestine all the time, one would expect that the bacterial cells will not make the required enzymes all the time. What has been noticed is that synthesis of the enzymes is quite tightly regulated. When these cells are grown in a culture medium lacking lactose, they made only traces of B-galactosidase enzyme. When some lactose is added to the culture, there is a sharp rise in the amount of B-galactosidase produced: within a few minutes of adding the

Contd...P/6.

inducer (lactose), the enzyme synthesis shot up over a thousand fold! This phenomenon is called enzyme induction.

Lactose metabolism in E. coli requires two enzymes: B-galactoside permease, which transports lactose into the cell, and B-galactosidase, which hydrolyzes lactose into galactose and glucose. The wild-type cells produce these enzymes only in the presence of lactose, and these cells constitute an inductive strain. When lactose is absent, then only minute traces of the two enzymes are produced by these cells. However, certain mutant strains are able to synthesize the two enzymes continuously irrespective of whether or not lactose is available. These are called constitutive strains. Enzymes which are produced only when their substrate is present are called inducible enzymes. Although inducer are quite specific, their structural analogues can also sometime act in the same way.

The lactose system can be analyzed by isolating two types of mutants, lac Z and lac Y, that are defective in B-galactosidase and galactoside permease respectively. The constitutive mutants cannot regulate themselves and continue to produce the two enzymes at all times; these mutants are designated lac I as they are very different from lac Z or lac Y. Lac I gene is located very near the lac Z gene in the bacterial chromosome (Fig.).

The above description should not imply that it is only the lactose metabolism that is regulated. In fact, various other metabolic systems of bacterial cells are also regulated. Examples include the enzymes of the biosynthetic routes for amino acids and nucleotides. However, there is one very notable difference between the patterns of lactose regulation and amino acid or nucleotide regulation: the enzymes in the later case are not inducible but repressible. That is, they are normally synthesized at high levels but if the end product of one of these biosynthetic routes is added to the culture suspension, the enzymes that synthesize it are repressed (i.e. inhibited) to a quite low level. For example, addition of tryptophan will repress the enzymes involved in tryptophan synthesis.

In general we may state that catabolic enzymes are usually inducible by their substrates while biosynthetic enzymes are usually repressible by their end products.

Contd...P/7.

-: 7 :-

In the above examples the genes Z and Y which produce β-galactosidase and galactoside permease, are called structural genes. Inducible activity of these structural genes only occurs if a normal O-gene called the operator gene is present to the left of the Z-gene (Fig.). The combination of the operator gene and the structural genes is called an operon. In the system studied by Jacob and Monod, there were actually three structural genes (Z, Y and a) and one operator (O) (a stands for thiogalactoside transacetylase). So, all these four genes together made up a single operon, called the lactose operon.

In the constitutive mutants, the gene i mapped some distance away from the O-site, and its site was called the regulator or inhibitor site (i). The normal product of this i gene is a repressor molecule which binds to the normal operator and switched it off when the inducer (lactose) was not present. On the other hand, when the inducer is available, the repressor is preferentially bound to the inducer, and hence the operator continues to remain switched on (Fig.).

An operon consisted of a system of cistrons, operator, and promoter sites. The regulator (i) plus the operon together determine the inductive or constitutive production of the protein products of the structural genes of any particular operon. The complete sequence for the lactose system may be written as :

i p o z y a

--XOX--

PROTEIN SYNTHESIS

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Proteins constitute more than half the total dry weight of a cell and their synthesis depends on the collaboration of several classes of RNA molecules and requires a series of preparatory steps. First, a molecule of messenger RNA (mRNA) must be copied from the DNA that encodes the protein to be synthesised. Meanwhile, in the cytoplasm, each of the 20 amino acids from which the protein is to be built must be attached to its specific transfer RNA (tRNA) molecule, the subunits of the ribosome on which the new protein is to be made must be preloaded with the auxiliary protein factors. Protein synthesis now begins when all of these components come together in the cytoplasm to form a functional ribosome. As a single molecule of mRNA moves stepwise through each ribosome, the sequence of Nucleotede in the mRNA Molecules is translated into a corresponding sequence of amino acids to produce a distinctive protein chain.

RNA Polymerase Copies DNA into RNA : the process of DNA transcription

RNA synthesized on a DNA template in a process known as DNA transcription. Transcription generates the mRNA that carries the information for protein synthesis, as well as the transfer, ribosomal and other RNA molecules that have structural and catalytic functions. These RNA molecules are synthesised by RNA polymerase enzymes, which make on RNA copy on a DNA sequence. In eukaryotes , different RNA polymerase molecules synthesize different types of RNA, but in bacteria a single species of the enzyme mediates all RNA synthesis.

The bacterial RNA polymerase is a large multi-subunits enzyme associated with several additional protein subunits that enter and leave the polymerase - DNA complex at different stages of transcription. Free RNA polymerase molecules collide randomly with the chromosuom, sticking only weakly to most DNA, However, the polymerase binds very tightly when it collides with a specific DNA sequence, called the promoter, that contains the start site for RNA synthesis and signals where RNA synthesis should begin. After binding with the promotor, the RNA polymerase opens up a local region of the double helix to expose the nucleotides on a short stretch

Contd...P/2.

of DNA on each strand. One of the two exposed DNA strands acts as a template for complementary base-pairing with incoming ribonucleoside triphosphate Monomers, two of which are joined together by the/polymerase to begin RNA chain. The RNA polymerase molecule then moves stepwise along the DNA unwinding the DNA helix just ahead to expose a new region of template for complementary base pairing. In this way the growing RNA chain is extended by one nucleotide at a time in the 5' to 3' direction. The chain elongation process continues until the enzyme encounters a second special sequence in DNA, the termination signal, at which point the polymerase releases both the DNA template and the newly made RNA chain.

The Promoter Sequence Defines Which DNA Structure is to be Transcribed:

In principle, any region of the DNA double helix could be copied into two different mRNA molecules - one from each of the two DNA stands. In reality only one strand is use-d as a template in each region, and the RNA made is therefore similar to the nucleotide sequence of the opposite, non-template DNA strand, Which of the two strands is copied depends on the promoter, which is an oriented DNA sequence that points the RNA polymerase in one direction or the other. Because RNA chains are synthesized only in the 5' to 3' direction, the promoter determines which DNA strand is copied. The DNA strand that is copied into RNA is often different for neighbouring genes, as illustrated for a small region of a chromosome.

DNA footprinting studies indicate that the E_ coli RNA-polymerase covers a large region of DNA when it binds to a promoter, the polymerase "footprint" is about 60 pairs long, extending about 40 base pairs "upstream" and 20 base pairs "downstream" from the start site for transcription. Comparison of the DNA Sequences of many strong promoters reveals that the polymerase primarily recognises two highly conserved DNA sequences, each six nucleotides long, which are located upstream from the start site and separated from each other by about 17 nucleaotides of unrecognised DNA:

Contd...P/

Conserved sequences found in all examples of a particular type of regulatory region in DNA, such as promoters are called consensus sequences. e.g. in a large number of E. Coli promoters the two consensus hexanucleotide sequences are TTGACA and TATATT.

Eukaryotic cells have three different RNA polymerases. One of these makes all of the RNAs that codes for proteins (i.e. the mRNA); the other two make RNA molecules with structural and catalytic roles (such as ribosomal RNAs and tRNAs). All three large multisubunits enzymes that resemble the bacterial enzyme, but the promoters each enzyme recognises are more complex and not as well characterised. It is unclear why both bacterial and eukaryotic RNA polymerases are such complicated molecules with multiple subunits and a total mass of more than 500,000 daltons,

TRANSLATION :

We now begin to look into how single-stranded RNA molecules function during the process of translation, or protein synthesis.

Transfer RNA Molecules Act as Adaptoors that translate Nucleotide Sequences into Protein Sequences.

All cells contain a set of tRNAs, each of which is a small RNA molecule (most have a length of 70 to 90 nucleotides). The tRNAs by binding at one end to a specific codon in the mRNA and at their other end to the amino acid specific by that codon, enable amino acid to live up according to the sequence of nucleotides in the mRNA. Each tRNA is designed to carry only one of the 20 amino acids used for proteen syntesis : a tRNA that carries glycino is designated as tRNA Gly, and so on. Each of the 20 amino acids has at least one type of RNA assigned to it, and most have several. Before an amino acid is incorporated into a proteen chain, it is attached by its carbonyl end to the 3' end of an appropriate tRNA molecule. This attachement serves two purposes. First, it links

Contd...P/4.

cavaleatly the amino acid to a tRNA containing the correct anti codon. Codon - anticodon pairings enable each amino acid to be inserted into a growing protein chain according to the dictates of the sequence of nucleotides in mRNA thereby allowing the genetic code to be used to translate nucleotide sequences into ~~protein~~ protein sequences. The second function of the amino acid tRNA attachment is to activate the amino acid by generating a high energy linkage at its carbonyl end so that it can react with the amino ~~ax~~ group of the next amino acid in the sequence to form a peptide bond. Non-activated amino acids cannot be added directly to a growing polypeptide chain.

Specific enzymes couple Each Amino Acids to its appropriate tRNA Molecule.

Only the tRNA molecule and not its attached amino acid determines where the amino acid is added during protein synthesis. A tRNA finds with its appropriate amino acid out of the twenty different amino acids with the help of the enzyme aminoacyl-tRNA synthetase. There is a different synthetase enzyme for each amino acid (20 synthetase in all).

Initiation Process:

During the initiation phase of protein synthesis, the two subunits of the ribosomes are brought together at the exact spot on the mRNA where the polypeptide chain is to begin. The small subunit is initially loaded with initiation factors (Fig.) and finds the start condon AUG. Then the large subunit is attached. A special tRNA which carries methionine binds to the condon. The metheonine tRNA (formyl methione in prokaryotes) occupies the P-site another aminoacyl tRNA depending on the next condon comes and attaches to the A-site. Amino acids are added to the carbonyl-Terminal end of a growing polypeptide chain. The fundamental ~~x~~ reaction of protein synthesis is the formation of a dipetide bond between the carboxyl group at the end of a growing polypeptide chain and a free amino group on an amino acid. Throughout the entire process the growing carboxyl end of the polypeptide chain remains activated by its covalent attachment to a tRNA molecule (a peptidyl tRNA molecule).

The Genetic Code:

In the course of synthesis the translation machinery (i.e. ribosome alongwith tRNA) moves in the 5' to 3' direction along tho mRNA and the mRNA base sequence is read 3 nucleolides (1 triplet) at a time. Since RNA is constructed of 4 types of nucleotides (adenosine, guanosine, thymidine and uridine), there are 64 possible combinations of three nucleotides (Fig.) (4x4x4x4). Three of these (UAA, UGA and UAG) do not code for amino acids, instead they specify termination of polypeptide chain, they are known as stop signals. Thus there are 61 [illegible] codon to specify only 20 different amino acids (Fig.).

A ribosome moves stepwise along the mRNA chain:

A ribosome contains three binding sites for RNA molecules, one for mRNA and two for tRNAs. One site called the peptidyl site or P-site, holds the tRNA molecule that is linked to the growing end of polypeptide chain. Another site, called the aminoacyl-tRNA binding site or A-site, holds the incoming tRNA molecule charged with an amino acid. The process of polypeptide chain elongation on a ribosome can be considered as a cycle with three discrete steps. Step 1 - an amino acyl tRNA molecule binds to a vacant A site by forming base pair with a codon in mRNA. Step 2 - The carboxyl end of the polypeptide chain is detached from tRNA and gets linked to the amino end of the amino acyl tRNA at the A site. This reaction is catalysed by peptidyl transference enzyme. Step 3 the new peptidyl tRNA in the A-site is traurtreated to P-site by movement of the rebosome exactly by 3 nucleotides. This is an energy consuming process and requires GTP.

To the free A-site a new aminoacyl tRNA comes at binds and the above processes continue.

Termination:

Three of the 64 codons (UAA, UAG and UGA) in a mRNA molecul are stop codons, which terminate the translation process. Cytoplasmic protein called release factors bind directly to any stop codon that reaches A-site on the ribosome. This binding alters the activity of the enzyme peptidy transferase and causes the release of the newly formed polypeptide, into the cytoplasm. The ribosome then releases the mRNA molecule and dissociates into its two separate subunits, which can assemble again on another mRNA to begin a new round of protein synthesis.

TISSUE CULTURE

Mrs. P. Mishra.

Tissue culture is a general term that is concerned with the study of not only tissues, but also of cells, protoplasts and organs maintained or grown in vitro. In vitro technique (meaning literally in a glass) is relatively a new branch of biological science, is based on the concept of "Totipotency of living cells". a theory put forth by the German botanist Dr. G.Haberlandt in 1902. Totipotency may be defined as the inherent capacity of a living cell to regenerate into a whole organism and is derived from the fact that as each cell of an organism is derived from the fertilised egg it must possess the inherent capacity to divide and give rise to the whole organism. If all of the cells of a given organism are essentially and totipotent, then the cellular differences observed within an organism must arise from the responses of the cells to their microenvironment and to the other cells within the organism. It should be possible to restore suppressed functions by isolating the cells from those organismal influences responsible for their suppression. If these has been a loss of certain functions, so that the cells in the intact organism are no longer totipotent, then isolation would have no effect on restoring the lost activities. The use of culture techniques the scientist to segregate cells, tissues and organs from the parent organism for subsequent study as isolated biological units.

HISTORY:

In 1902 Habulandt was the first to attempt to cultivate isolated plant cells in vitro on an artificial medium .

In the 1930's the subject was put on a scientific foot through the works of Dr. R.J. in France and Dr. P.R. White in U.S.A. In the late 1950's, F.C. Steward and his Co-investigators at Cornell university demonstrated Cellular Totipotency. Their experiment consisted of culturing carrot root cells in a nutrient medium supplemented with coconut milk. The cells developed into plantlets.

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In 1963 Steward and 1964 Halperin and Wetherell demonstrated the production of thousands of somatic embryos from carrot cells plated on a nutrient medium in petridishes.

Totipotency of cells has now been repeatedly shown in a wide number of plant tissues of diverse origin. This unique property of cells has been imaginatively employed for propogating plants through tissue culture.

Broadly 2 kinds of plant growths are possible.

i) Organised growth: in which organised plant parts continue to grow or when organised structures are formed afresh from unorganised tissues.

ii) Unorganised growth: which is seldom found in nature. This occurs fairly frequently when pieces of plant parts are cultured in vitro. They can be increased in volume by sub-culture and can be maintained in solid or liquid media for long periods.

In practice the following kinds of cultures are most generally recognised

Callus Culture: Cell aggregates arise from disorganised growth of small plant organs or detached plant tissue or previously cultured cells.

Cell Culture: is the Culture of individual cells or populations of cells and cell clumps dispersed in an agitated liquid medium. Also known as Suspension cultures. Here the cells are no longer organised into tissues.

Protoplast culture: is the culture of plant cells that have been isolated without a cell wall.

Organ Culture : is the culture of whole organs of a plant in aseptic conditions. This may be meristem or shoot tip culture, single node culture, another culture embryo culture etc.

Tissue Culture: The maintenance of growth of tissues in vitro, in a way that may allow differentation and preservation of their architecture and/or function.

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Sub-Culture: Aseptic transfer of a part of a culture to a fresh medium.

Callus Disorganised meristematic or tumor like mass of plant cells formed under invitro conditions.

Explant A fragment of a tissue or organ which is taken from its original site and transfered to an artificial environment for growth or maintenance.

Passage : The duration of each sub culture.

Complete new plants can be derived from tissue culture in 3 ways.

i) from pre-existing shootbuds which are encouraged to grow and proliferate

ii) from shoot morphogenesis when new shoots are induced to grow from unorganised tissues or directly upon explanted tissues of the mother plant.

iii) through the formation of Somatic embryos which resemble the seed embryos of intact plants and which in the same way can grow into seedlings.

Ideally newly formed plants will be genetic carbon copies of parent plants but irregularities to sometimes occur.

The parts to be used as explants depend upon

i) the type of culture to be initiated.

ii) the purpose of the proposed culture

iii) the plant species to be used.

The correct choice of explants and the time of the year at which it is obtained can have an important effect on the success of tissue culture.

Plants growing in the external environment are invariably contaminated with micro-organisms. Such organisms in particular bacteria and fungi compete adversely with plant materials growing in vitro.

Explants must therefore be freed from contaminents before they are transferred to culture and the vessels and media in which cultures are grown must be sterilised and kept in aseptic condition throughout.

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There are a variety of chemical agents in common use for the surface sterilization of plant material. The choice of agent and the time of treatment depends on the sensitivity of the material to be sterilized. Frequently it is seen that too much of sterilization leads not only to the complete removal of all micro-organisms but is also lethal to the plant tissue. It is therefore, important to determine the optimal conditions for each tissue.

The sterilizing agent should be easily removable, because the retention of such chemicals will seriously affect the establishment of the collus. Repeated washing with distilled water will remove most chemicals. Some sterilizing agents breakdown and become less toxic and the products can be easily washed away. For Ex. Sodium hypochlorite breaks down to give chlorine, the active agent, and sodium hydroxide, which can be removed, while others like Hydrogen peroxide decompose to give harmless components which evaporate.

Following surface sterilization plant materials are transferred to suitable nutrient medium. The composition of the culture medium is an important factor in the successful establishment of a tissue culture. Culture conditions favouring callus growth may not be suitable for organ differentation. Each tissue type requires a different formulation depending on whether the objective is to obtain optimum growth rate or induce organogenesis.

Several media have been developed by various workers to suit particular requirements of a cultured tissue.

A standard or b ' medium consists of a balanced mixture of micronutrient and micronutrient elements (salts of chlorides, nitrates, sulphates, phosphates and iodides of Ca, Mg, K, Na, Fe, Mu, Zu and boron) Vitamins source organic growth factors (aminoacids, Urea and peptones) a source of reduced nitrogen supply and plant hormones.

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Addition of deproteinized coconut milk, tomato juice, water melon juice, orange juice and other plant extracts, YE, ME, or protein hydrolysate brings about mitosis in quiescent cells and has proved beneficial though not essential in cases where the tissues do not divide easily on a purely synthetic medium. Different nutrient media have been formulated by various workers-

Iron is added in the form of ferric citrate or as FeEDTA (ferric sodium - ethylene diamime-tetra acetate) in order to ensure its availability over a wide range of PH of the medium.

The PH of the nutrient solution which is a balanced mixture of macro and microelements is adjusted to 5.6-6.0 by the addition of 0.1 HCL or NaoH.

The growth regulator requirments for most Callus cultures are auxin and cytokinin. Auxins, a class of compounds that stimulate shoot cell elougation, resemble IAA in their spectrum of activity. Cytokinins, which promote cell division in plant tissue regulate growth and development in the same manner as kineti (6- furfuryl amino purine) cytokinins are mainly N^6- substituted aminopurine derivatives. Auxin-cytokimin supplements are instrumented in the regulation of cell division, cell elougation, cell differentation and organ formation. Gibberellins are rarely added to culture media, altho GA_3 has been used in apical meristem cultures (Morel and Muller 1964).

The auxins most frequently employed are IAA, α NAA (α-naphthalemacetic acid) and 2,4-D(2,4-dichlorophenoxyacetic acid). IBA (Indole-3 butyric acid) is a particularly effective sooting agent. IAA is a naturally occuring auxin, but unfortunately it is readily degraded by light and enzymatic oxidation. Because IAA oxidase may be present in cultured tissues, IAA is added to media in relatively high concentrations (1-30mg/l). The most effective auxin for callus proliferation for most cu cultures is 2.4-D. The most widely used cytokimins in culture media are kinetin, benzyladenine and Zeatin. Kenetin and benzyladenineare synthetic compounds, whereas Zeatin occurs naturally.

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Culture Techniques

The first techniques developed for callus culture were simple and employed media solidified wilt *agar*, *gelatin* or *silica gel*. The ~~q~~ great merit of this form of culture is its extreme simplicity. Only simple standard laboratory glasswar is required and there is no need for complex mechanical devices or elaborate containers. In addition large numbers of cultures can be accomodated in a small space. However, a survey of the literature on plant tissue cultures will show that solid media have now been largely relegated to the establishment and maintenance of callus cultures. Much of th recent critical work on nutrition metabolism and growth has been performed with liquid media. The reason for this are th limitations one comes across when culturing on a solid media. Firstly, only one part of the callus or explant is in contact with the surface of the medium. It is likely, therefore, that as culture proceeds, inequalities in the growth response will arise in response to the nutrient gradients set up between callus and medium. Similarly there may be gradients in the exchange of respiratory gases due to occlusion of the base of the explant. Gradients of toxic waste products may be establis ed. A further disadvantage observed in that cultures grown on solid media cannot be transferred to liquid media without som disturbance to the tissue. Despite these limitations the culture of callus on solid media with agar remains the method par excellence for the routine maintenance of cultures and is still used for experimental investigations.

The culture of tissues in unshaken liquid media has all the advantages of the solid medium methods and many of the disadvantages are absent. Here the tissue is placed on an ash-less filter paper ~~sppp~~ support held at the interface of the medium wilt the air in the test tube. The filter paper acts as a wick providing nutrient while keeping the tissue in the gas phase.

The culture of explants agitated in a liquid medium eliminates many of the disadvantages ascribed to the culture of tissues in stationary culture. Movement of the tissue in relation to the nutrient medium facilitates gaseous exchange, removes polarization of the tissue due to gravity, and eliminates nutrients gradients within the medium and at the surface of the tissue. These can be placed under two categori

1) Continuous immersion: techniques in which the tissue is always in contact with the culture medium and the mixture is shaken or stirred continuously.

2) Periodic immersion: Techniques in which the tissue spends periods immersed in the liquid medium alternating with period in air. Such an arrangement ensures adequate mixing as well as providing efficient gaseous exchange.

Callus cultures require to be transferred periodically to a fresh nutrient medium. Extensive growth leads to the exhaustion of nutrients, drying out of solid media or concentration by evaporation of liquid media and the accumulation of tissue metabolish. Cultures maintained on agar at 25°C or above require to be sub-cultured every 4 to 6 weeks. In the early stages of callus development it may be convenient to transfer the whole piece of tissue but the sub-culture of established callus demands the frequent ~~such~~ sub-division and transfer of separated pieces. In this case it is important to transfer small healthy looking pieces to the surface of fresh agar medium. Failure to transfer cultures ultimately leads to the death of the callus ~~which~~ while a sub-culture from necrotic callus tends to grow much less actively than one taken from an actively growing healthy culture.

The general growth characteristics of a callus involve a complex relationship between the plant material used to initiate the callus, the composition of the medium and the environmental conditions during the incubation period. Establishment of a callus from the explant can be divided roughly into 3 developmental stages (i) induction (ii) cell division and iii) differentiation. During the initial induction phase metabolism is stimulated as the cells prepare for division. The length of this phase depends mainly on the physiological status of the explant cells as well as the cultural conditions. Subsequently, there is a phase of active cell division as the explant cells revert to a meristematic or "dedifferentiated" state. The third phase involves the appearance of cellular differentiation and the expression of certain metabolic pathways.

A homogeneous callus consisting entirely of parenchyma cell is rarely formed. Cytodiffrentiation occurs in the form of ~~traceh~~ tracheary elements, sieve elements, suberised cells, secretory cells and trichomus. Small nests of dividing cel form "meristemoid" or vascular nodule that may become cent for the formation of shoot apices, root primordia or roci embryos. One serious problem associated with the use of ca cultures, as well as other cell culture systems, is geneti instability resulting in variations in phenotypes within t cell population. Phenotypic variations arising during cult may have either a developmental (epigenetic) or a genetic basis. Genetic variations may involve chromosomal aberrat nuclear fragmentation and endo duplication resulting in polyploidy. The frequency of these nuclear abnormal usually increases with the age of the cylture, and the cul ral conditions may act in a selective manner. Certain ancu or polyploid cells might gain an advantage in division rat over the normal cells proliferate to a greater extent, and ultimately remove the dominant cell line of the culture.

The first indication that in vitro organogenesis coul be chemically regulated to some extent was given by Skoog (1944). He found that the addition of auxin to the medium served to stimulate root formation, whereas shoot initiati was inhibited. Subsequently it was found that adenine sulpha was active in promoting shoot initiation and this chemical reversed the inhibitory effect of auxin. The studies of skoo and his clleagues led to the hypothesis ~~of~~ that organogenes is controlled by a balance between cytokinis and Auxin. A relatively high auxin:cytokinis ratio induced root formati in tobacco callus, whereas a low ratio of the same hormones favoured shoot. The formation of floral buds, vegetative buds, and roots has been demonstrated in thin cell-layer explants of several species by regulating the auxin:cytoki ratio, carbohydrate supply and environmental conditions.

Torrey(1966) - Organogenesis in callus starts with th formations of clusters of meristomatic cells (meristermoids capable of responding to factors within the system to prod a primordium. Depending on the nature of the internal fact the stimuli can initiate either a root, a shoot or an embryoid. Many observations on organ formation in cultured tissues support the hypothesis that localised meristematic activity precedes the organised development of roots and shoots. The factors that regulate the origin of these meristematic zones are not understood. Since these zones a located in the vicinity of the tissue-medium interface, it has been suggested that physiological gradients of substances diffusing from the medium into the tissue may play a role in determining the loci at which meristemoids are formed.

Once the cultures are established these are sub-cultured for the increase in volume. The duration of each subculture is called a passage. Sub-culture is usually done at interval of 4-6 weeks, length of which depends upon the rate of growth.

Plant propagation through tissue culture also termed as micropropagation which is generally divided into four stages:

Stage O - mother plant selection and preparation
Stage I - establishing an aseptic culture
State II- Production of suitable propagular
Stage III- Preparation for growth in the natural environment.

Micropropagation is usually very much more rapid than the traditional methods. It cannot only provide increased rate of propagation but can facilitate vegetative nultiplication of plants that had previously been proved difficult or impossible to propagate. The efficiency and reliability of vegetative propagation through micropropagation is important for a number of reasons.

In the past, agronomists tailored the environment to suit the crop but plant breeders have to tailor the crop to suit the environment. Much has been and continues to be accomplished by the use of conventional, non-molecular tool of genetics. We have yet to exhaust the potential of classical genetics to improve crop productivity. But with increasing pressure for further improvements in Crop plants, crossability barrier, linkages, variability in gene expression, low selection efficiency for complex traits etc., pose limitations of conventional breeding techniques effective use in the future. The new tools of genetics which include tissue culture techniques offer new possibilities in solving some of these problems.

Meristem, Shoot-Tip and Bud culture:

These techniques are alternative means of asexual propagation of economically important plants. The explant of meristem culture may either be the apical dome (apical meristem) or more frequently, the apical dome plus a few sub-adjacent leaf primordia. The cultures are initiated from either terminal or axillary buds usually with the stem segment attached, using either a growing or dormant shoot. Plants derived from meristem, shoot tip and bud cultures are generally phenotypically homogenous then indicating genetic stability. Application of these tissue culture techniques for rapid cloral propagation is highly desirable in cases such as (a) problem sps. - do not produce seeds or produce very few seeds, produce viable seeds, have slow rate of multiplication. When advanced breeding lines have been identified it is important to propagate as many seeds or plants as is possible to permit variety release. At this point tissue culture may be used in conjuction with other asexual methods to propgate clonal lines for seed production. Such an approach was used in Equador to establish elite clones of Pyrethrum(Chrysathemum cinerariaefolium's) for commercial production. In some cases cultivated crops, particularly eash crops, where each individual plant is valuable, may be cloned using tissue culture.

Plants obtained through conventional vegetative propagation are liable to accumulate systematic viral, bacterial or fungal infections, disease free individuals obtainable only in small numbers will similarly need rapid multiplication.

It has been observed in a large number of plant species that the concentration of infective viruses is low in the apical meristem of a plant. The lack of vascular differentiation in the meristem impaires intercellular movement of viruses and the active metabolism of mitotic cells precludes viral infection. In certain crops or ornamental species, it has been economically useful to develop in vitro techniques to produce virus - eradicated plants. These species include the elimination of mottle virus from straw beery, potato virus x from potato, cauliflowers mosaic virus from cauliflowers.

Plants regenerated from callus may also be free of viral infection. Callus tissue is similar to meristematic tissue as cell mutosis is rapid and vascular differentiation is incomplete. Virus-free plants have been regenerated from callus in tobacco, geramium, Gladiolus and potatoes. Although callus tissue results in virus-free plants, chromosome abnormalities have been documented in plants regenerated from callus tissue.

Anther Culture:

The advantage of the in vitro production of haploids over the conventional method is the shortenning of time to achieve homozygosity. Since homozygosity is attained in one step, one to 5 years of selfing and reselection which is done in curcumtienal breeding could be bypassed. Since no further segregation occurs each plant derived from anther culture is a potential new variety and could be screened immediately for desirable characters. Koreawas able to release an anther cultured derived variety (Hwas embryo) in 5 years, in contrast to ten or more years required to develop a variety through conventional means. Rubber trees from selected pollen plants with increased yields of latex, superior growth vigor, early bloom, and increased cold resistance were also reported. Likewise,winter bread wheat 'Florin' has been released as a commercial winter wheat variety. It is the first wheat variety developed by anther culture method in the Western World.

Anther culture also allows faster production and selection of useful mutants. Since haploids possess only are set of allles at each lacus, it is possible for recessive mutants to be detected. In this way, difficulties in the selection of mutants due to dominance are negligible and most, if not all genes, are expressed. This allows easy isolation of recessive mutations because they are expressed immediately. Although in anther culture as in mutation breeding it is not difficult to generate variants and most will be deleterious by virtue of some metabolic imbalance which may be expressed as reduced vigour or fertility, rice, plants with larger seeds, higher levels of seed protein, shorter statue and more highly tillred than the cultivar were obtained from selfed anther-derived plants.

Cell culture:

Many secondary plant products such as dyes, fragrances and drugs are produced by the plants often in very minute quantities as defense mechanism for their survival.

In vitro culture of different explants could be helpful in isolating and increasing the yield of sp. plant cells which produce such compounds. Moreover, in vitro culture yield products which are more easily purified. The production of secondary plant products has been achieved by especially by selecting high-producing cell lines either by visual selection as in the case of pigmented products or by chemical analysis of the cells as in the case of colourless compounds.

Shikonim , a red naphthoquinone pigment used for medicine, dye and cosmetics is isolated from the purple root of Lithospermum erythrorhizor, a perennial herb native to Japan, Korea and China. Japnese scientists have devised a cell culture system in which the cells containing the red pigment can grow faster and yield higher compared with the whole plant. Other important secondary plant products are Vinblastine and Vincristine which are isolated from Catharanthus roseus. Berberine are important pharmaceutical alkaloid that has antibacterial and anti-inflammatory activities (12.7%) from the roots of coptis japanica (2.4%) more than 5 years). Yield of digitoxin which is the most active principle of Digitatis, a powerful cardiac stimulant and directive, can be increased by as much as 400% in tissue cultured with GA3.

Cell cultures which could be induced to form plants through somatic embryogenesis have several advantages. This is extremely important in the exploitation of hybrid vigour in crops. FI hybrids may yield more than either parent-better known as hybrid vigour. The constraint in the use of hybrid technology is the high price and limited supply of hybrid seeds. An alternative is the mass production of encapsulated somatic embryos from FI seeds.

Somacloral variation which refers to the genetic variability observed in plants, and their progeny from tissue and cell cultures could be utilised in increasing crop productivity. Somacloral variation has been observed in economically important plant sps. which include tomato, maize, potato aljalja tobacco, rice etc.

Plants regenerated from protoplasts of the potato variety Russelt Burbank were not precise carbon copies of the parent. Shepord(1982) examined over 10,000 plants regenerated from protoplasts and demonstrated that each differed in some way from the parent. The protoclomes showed variation in tuber colours, weight, specific gravity length, width and height, maturity date and photoperiod requirements. A few were more resistant to Alternania Solari toxin than the parent. and some should field resistance to early blight About 2.5% of protoclones screened were resistance to *phytophthora infestans*.

Embryo Resource

Interspecific hybridization is an important tool for introducing valuable trails from wild species into the gene pools of cultivated plant species. Usually, such crosses do not yield any agriculturally beneficial hybrids primarily because in the transfer of beneficial alien genes across interspecific or intergeneric barriers, too many deterious genes are also introduced. The resulting disturbance in equilibrium between the growth processes of the maternal tissues, embryo and endosperm lead to embryo mortality and seed collapse. These post zygotic in compatibilities can be overcome by embryo culture or embryo rescue.

Embryo rescue has been useful in the hybridization between two jutespecies *Corchorus olitorcinus* and *C. capisularis*. Here improved quality with resiatance to pest and diseases were incorporated on one genotype. Wide hybridization is now being utilised in rice. Genes for brown planthopper resiatance have been transferred from the wild sps. O. officinialis into cultivated rice by Embryo rescue.

Synthetic seeds:

Somatic embryo are of typical bipolar nature and resemble the zygotic embryos found in the seed. Now-a-days, biotechnologists are working on the possibilities of producing artificial or synthetic seeds from somatic embryos of important agricultural and forest plants. In "Synthetic seed", a somatic embryo is generally encapsulated in some nutrient gel which acta as the endosperm for the somatic embryo. These synthetic seeds can be stored and transferred easily and can be sown in the field beds directly.

Protoplast Isolation and Fusion:

Somatic hybridization which can be accomplished by protoplast fusion is a means of overcoming prezygotic incompatibilities in interspecific, intergeneic, and interfamilial crosses. This technique is also important in the transfer of cytoplasmic traits such as male sterility and herbicide tolerance.

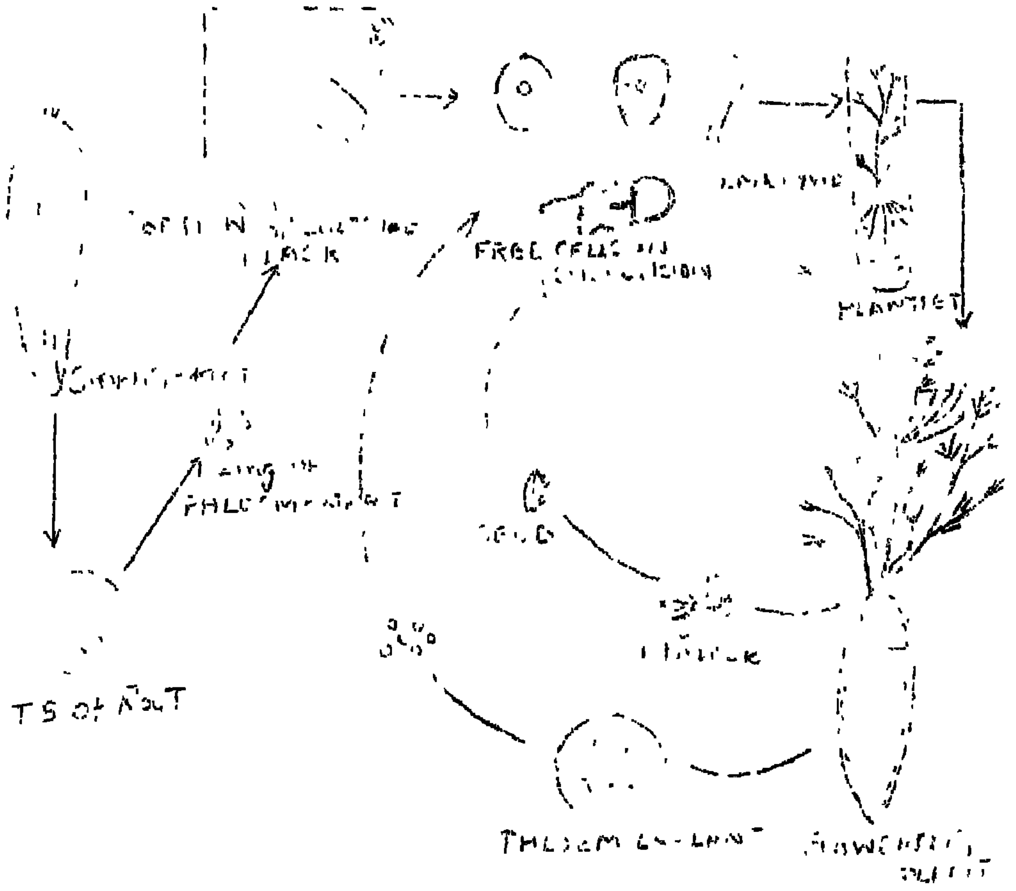
Protoplast fusion is made possible by the use of polyethylene glycol or by electro-fusion. Fused protoplast when properly selected and regenerated into plants may exhibit combined characteristics of both . There are some successful somatic hybrids obtained from protoplast fusions in *Brassica*, *Nicotiana* and *Solanum*. Studies in the transfer of resistance to bacterial wild fire diseases and fungal shank from *Nicotiana rustica* into *Nicotiana tabacum* were attempted usin somatic hybridization (Patrik et al 1982).

Recombinant DNA Transfer

This technique involves the identification of the desired genes, isolation, duplication and insertion into a receipient cell. The goal is not only to insert the gene, but to have the gene expressed.

The transfer of genes should be done by vectors which ca the desirable gene. The most promising vectors so far seems t be the tumour inducing(Ti) plasmid . carried by *Agrobacterium tumefaciens*. This bacterium causes tumour growths around the root crowns of plants. It infects only dicotyledonous plants and its virulence is due to the Ti plasmid, which when it is transferred to plant cells induce tumours. Once inside the cell, a smaller segment of the Ti plasmid, called T-DNA, is actually incorporated into the chromosomes.

Since *A. tumefaciens* does not infect important cereals such as maize, rice etc. other gene transfer techniques that are potentially applicable to all sps. are being developed. One of these techniques is electroporation which uses brief, high voltage electric shocks of few microseconds in duration, which opens pores in the membranes of plants protoplasts thereby facilitating the introduction of plasmids carrying desirable genes.



PLANT CELLS ARE TOTIPOTENT

PROSPECT OF PLANT PROTOPLAST TECHNIQUE FOR GENETIC MANIPULATION

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Plant cell differs from animal cell by possessing a cell wall which is made up of cellulose. When the cell wall is removed, the wall less cell is called as protoplast. This protoplast is perceived when plasmolysis occurs in a plant cell. Ever since the protoplasts were recognised, attempts had been made to isolate them from plant tissues and induce fusion in them. Klerker (1892) was successful in cutting the cell wall in leaf tissue plasmolysed in 0.4 M potassium nitrate and through controlled deplasmolysis he was able to release the protoplasts into the medium. Kuster (1910) was the first to achieve fusion between sub-protoplasts as well as the freely isolated plant protoplasts. Michel (1937) standardised the technique and used light microscopic markers to induce anto-plastic (between sub-protoplasts), homoplastic (protoplasts of the same species) and heteroplastic (protoplasts of different species or geneva) fusions. Hofmeister (1954) induced fusion in plant protoplasts by suspending them in isotonic solution such as sea water. However, none of these methods was efficient or reproducible.

The discovery of the chance fusion of two mouse tumor cell lines grown together in the same culture plate by Barski et al. (1960) opened up the prospect of animal cell fusion even in remote cell lines like mouse and man. Such hybrid cells were not mere curiosities since there were preferential loss of chromosomes of different parental lines in the hybrid cells. It came as a handy tool for genetic analysis and gene mapping in higher animals including man.

Almost simultaneously in the 1960's. enzymatic methods for large scale production of protoplasts in plants were discovered.

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Take-be et al. (1968) developed the procedure of sequential engyme application by treating plant tissue with pectinase for maceration and celulase for cell wall degradation respectively. On the other hand, power and cocking (1968 introduced mixed engyme approach in which plant cells were plasmolysed in the presence of mixtures of pectinase and cellulase. In such procedures, when isolated, protoplasts some times undergo spontaneous fusion. However, in mixed engyme method, fusion within the sympla m became more frequent due to the plasmodesmatal connections. The frequency of such undesirable spontaneous fusion was prevented either following sequential engyme procedure or plasmolysing the cells prior to mixed engyme treatment there by severing the plasmodesmatal connections. Though the spontaneous fusion bodies arising from such homokaryotic fusion were of no significance the observation that cell wall is automatically formed around such fused protoplasts and the two nuclie normally fuse and divide in them, generated hope for fusing protoplasts of different species of plants. With the success of culturing protoplasts, raising callus from them and regenerating whole plants from such calli opened a new method of plant hybridization through the fusion of somatic cells. Since the incompatibility barrier, such as the prevention of key steps in pollination, pollen tube development, fertilization and embrgo or endosperm development encountered in sexual hybridization between remote species was found to be nonexistent in somatic hybridigation, success could be achieved soon not only in fusing cells derived from taxonomically distinct plants but but ever bringing about inter-kingdom fusion between plant and animal cells.

The success of somatic hybridigation depended upon the efficient fusion between protoplasts grown in culture and soon fusion methods were standardsed by employing different fusogenic agents. Power et al. (1970) using sodium nitrate brought about fusion of protoplasts which was successfully employed in many protoplast systems. Keller and Melchers (1973) used mannitol solutions containing calcium ions buffered at high PH (10.5) and this method is

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now found to be very efficient in widely diverse kinds of protoplast systems. Kao and Michayluk (1974) assessed the ability of polyethylene glycol (PEG) which was though found to have moderate levels of fusion could be applied to a wide range of plant and animal organisms. PEG and Ca^{+2}/high pH could also be used together and even in a veyy "small scale fusion", higher efficiency was recorded for both the agents (Patnaik, 1987). In addition to these three agents, several chemical compounds have now been tested and as many as 20 compounds are now identified which promote cell hybridigation with nearly same efficiency as PEG (Klebe and Mancuso, 1981).

As alternate to chemically induced protoplast fusion Zimmermann and Scheurich (1981) introduced a technique of high frequency fusion of plant protoplasts by electric fields. This was described as a two step process. First, the membranes of neighbouring protoplasts are brought into contact with a nonuniform AC field in a process called dielectrophoresis. Protoplasts, thus in transient dipoles aggregate between the electrode in the form of "Pearl Chains". The points of contact between the plasma membranes of the neighbouring protoplasts are broken down electrically with a short DC pulse. Membrane reorganisation following the DC pulse results in cell fusion.

Though the exact mechanism of membrane fusion is still debatable, some suggestions have been advanced mostly on the basis of chemically induced fusion. Ahkong et al.(1975) held the view that exogenous chemical agents could induce perturbation of the membrane bilayer with increased fluidity of the lipid region which would allow aggregation of inter-membraneous protein particles at places. When the membranes are closely opposed at regions where proteins are now absent, intermixing of the disturbed lipid molecules results in the coalescence of the adjacent bilayers. As regards the role of chemical furogens described, they are believed to play the role of establishing intimate contact between neighbouring membranes, alter surface negative charge of the membranes, trigger changes in membrane structure and permeability resulting fusion.

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With the development of technique of enzymatic isolation of protoplasts, their culture and regeneration of whole plants, a new tool could be available for genetic manipulation. While protoplasts as such could be amenable for the entry of exogenous genetic materials, and even cell organelles resulting in genetic transformation, fusion of protoplasts opened a new vista for bringing together widely diverse kinds of genomes otherwise not possible through sexual breeding. The aspect of somatic hybridigation may be many fold : (i) synthesis of amphidiploids between sexually incompatible species, (ii) producing heterozygous lines within the same species which ordinarily reproduce by vegetative means, (iii) transfer of part of a nuclear genome to another through chromosome elimination, (iv) formation of cytoplasmic hybrids (cybrids) to transfer cytoplasmic male sterility to a different line or species or (v) organelle transfer which is more readily achieved through fusion rather than direct uptake.

The major problem, after the fusion is induced between genetically different lines is the isolation or selective growth of the heterokaryons in the midst of parental cell population and homokaryotic fusion products. This could be achieved by precisely picking up the heterokaryons by micromanipulators or isolation through more sophisticated florescence flow cytometer or cell sorter and to grow them in nurse culture. But the selective method of culture for heterokaryons is more widely used. This is achieved by standardising culture media which is only conducive to the growth of the somatic hybrid cells but not to the parental cells lines. This type of selective method becomes possible when double-albino, auxotrophic and drug or antibiotic resistant mutants are available and wild types are formed by complementation through hybridization which are automatically selected. However, in the absence of such double mutants, half selective method is taken recourse to and in quite a number of cases, hybrid colonies are easily identified in a mixed culture. Basically for the identification of two parental cell lines in the fusion process chlorophyllose mesophyll

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protoplasts (fluorescing red under UV) are directly taken from the leaves of one parent and colourless protoplasts (florescing green under UV if treated with FDA or FITC) taken from cell suspension of other parent maintained for long time through cell passages. After the hybrid colonies are isolated they are grown separately and plantlet regeneration is induced following the basic techinques of tissue culture. When full fledged plants from the fusion products are raised and they bear flowers and fruits, various tests such as morphological, cytological and biochemical are carried out to prove the authenticity of their hybridity. Quite often fused products may not yield hybrid plants due to break-down at various stages.

Though a few years back somatic hybridization experiments were limited only to certain model plant systems predominantly from *Nicotiana, Petunia, Solanum, Datura, Daucus* (Schieder, 1982) etc. gradually these are being extended to other plant genera such as *Lycopersicon, Oxyza* etc.

- 0 -

Complete Review

THE TISSUES OF THE PLANT BODY

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Meristems :-

As a general rule, one of the obvious differences between plants and animals is the pattern of growth by which they attain their adult forms. Animals grow until they reach physical maturity, and during this time growth occurs throughout the body. Plants, however, continue to grow until they die but, as soon as the embryonic stage is passed, growth is restricted to certain regions called meristems (Fig.3.1). Here the cells remain embryonic and continue to divide, whereas in the rest of the plant they reach maturity and assume a permanent form. One type of meristem is always present at the tip of every root and stem. The activity of such apical meristems is responsible for the increase in length of the plant body and, in the case of shoot meristems, for the production of lateral branches, leaves and flowers. The growth initiated by apical meristems is known as primary growth, and all tissues formed from apical meristems are called primary tissues. In some plants, notably grasses, the increase in length of the stem is also due to the division of meristematic cells located at the base of each node and leaf sheath. These meristematic regions are called intercalary meristems (Fig.3.1b) because they are inserted or intercalated between mature primary tissues both above and below them. They are really portions of the apical meristem which become separated from the main body of the meristem and are left behind as the apex grows forward. Intercalary meristems may remain active long after the cells of the internodes above them have fully matured. Growth of the cells produced by intercalary meristems is responsible for the rapid elongation of the stem which often occurs just before flowering. The tissues formed from intercalary meristems are similar to the adjacent tissues derived from the apical meristem and are therefore classified as primary tissues.

Contd...P/2.

Some plants, especially monocotyledons, complete their life cycle by primary growth. In other plants, including most dicotyledons, the stem and root increase in thickness by means of a process called secondary growth which is initiated by lateral meristems or cambia (singular, cambium). These develop within the already existing primary tissues of the root and stem, and form secondary tissues in planes parallel with the surface of these organs (Fig. 3.1a). There are two cambia that may develop in a plant showing secondary growth, the vascular cambium and the phellogen (or cork cambium). The vascular cambium is responsible for most of the increase in thickness during secondary growth, whereas the phellogen produces a protective layer of periderm (or cork, This is formed in the outer region of the expanding root or stem when the primary surface layer (epidermis) is ruptured by the increase in thickness due to the activity of the vascular cambium.

Maturation of meristematic cells :-

Since all the cells of the adult plant body result from the activity of meristems, the question arises as to how meristematic cells are converted into mature cells. The essential characteristic of a meristematic region is that it consists of actively dividing cells which have the dual property of maintaining the meristem as a distinct region and, at the same time, of adding new cells to the rest of the plant body. In every meristem there are certain cells which divide in such a way that at each division one of the daughter cells (the initial) remains in the meristem, whereas the other daughter cell (the derivative) gradually passes out of the meristem and eventually becomes one or more cells within the main body of the plant. The stages by which a newly formed derivative reaches maturity can more easily be followed by reference to apical meristems rather than to lateral meristems.

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The meristematic cells at the apices of roots and stems (but not those of lateral meristems) have the characteristic appearance shown in Fig. 3.2. Although they appear approximately square in section, their basic shape is that of a 14-faced polyhedron, each face pentagonal. This is the shape assumed when a number of similar clastic spheres are subjected to pressure equally from all directions, until there are no air spaces between them. The cells have thin walls, the cell cavity is filled with dense cytoplasm and a large nucleus (up to two-thirds the diameter of the cell), and visible vacuoles are absent. During the process of becoming transformed into a mature cell, a derivative passes through three distinct but overlapping phases. The ability to divide is not confined to the initial cells but extends also to their immediate derivatives, which usually divide several times before starting to mature. The first phase in the growth of a derivative is therefore cell division, but this soon passes into the second phase, namely cell enlargement. Because the apical meristem constantly grows forward as a result of cell division, the newly formed derivatives come to occupy the region just behind the apex. It is in this subapical region that they begin to enlarge, and they continue to do so until by the time they reach maturity they are commonly many times larger than the meristematic cells from which they were derived (Fig. 3.2).

As a cell enters the phase of enlargement, droplets of cell sap form in the cytoplasm and these, after further increase in size, fuse to form several small vacuoles. The nucleus meanwhile remains suspended in the centre of the cell by strands of cytoplasm. The vacuoles continue to enlarge by uptake of water and finally coalesce to form a single central vacuole. As a consequence, the nucleus is moved to a peripheral position in the thin layer of cytoplasm lining the cell. While the vacuoles are expanding the whole cell increases in size but, despite the increase in surface area, the cell wall does not decrease in thickness because new wall material is continually being added to the original wall. Thus cell enlargement is not merely the inflation of a cell

Contd...P/4.

due to vacuolation, but it is an active process in which the amount of plant substance is increased as a result of intense biochemical activity by the cell.

In the process of assuming their mature form, cells not only increase in size but also become structurally modified to fulfil particular physiological functions in the adult plant. This modification for specialized functions is called differentiation (Fig. 3.3) and forms the third and last phase in the maturation of a meristematic cell. Differentiation starts while cells are still enlarging but is never complete until after they have ceased to enlarge. It should be regarded as the process by which cells become different not only from their meristematic precursors but also from their immediate neighbours. The extent to which a cell becomes differentiated depends on its final function. Some cells differ only slightly from meristematic cells (e.g. packing cells) while others become markedly different (e.g. elongate conducting cells). There are many ways in which plant cells can become specialized to serve particular functions, but most of them involve modifications of the cell wall. These modifications include such features as the deposition of a thick secondary wall, and the development of various types of pits.

Types of plant tissues :-

It should now be clear that the different types of cells in the adult plant are the product of the three overlapping processes of cell division, cell enlargement and cell differentiation. The resulting mature cells are not arranged at random but are associated in various ways to form recognizable groups called tissues. There are various ways of classifying tissues according to whether they are considered from a structural or functional point of view. Perhaps the simplest classification, and the one that will be followed here, is to divide tissues into two categories, simple and complex, on the basis of whether they consist of only one or more than one type of cell.

Contd...P/5.

Simple tissues :-

There are three simple tissues, parenchyma, collenchyma, and sclerenchyma. These terms are also applied to individual cells showing the characters of these tissues. Thus a parenchyma cell is not necessarily a unit of the simple tissue parenchyma, but it may also be a component of a complex tissue.

Parenchyma

This is the simplest type of mature tissue, being less modified from meristematic cells than any other tissue. Parenchyma cells are living cells which are sufficiently unspecialized to be capable of reverting to the meristematic condition. It is often difficult to draw sharp lines between tissues because cell types sometimes merge into one another. Parenchyma, in particular, has very ill-defined limits and covers a range of cells which differ widely in structure and function. It is thus impossible to say that a parenchyma cell will inevitably have certain features apart from the very general one of being alive at functional maturity. However, 'typical' parenchyma cells (Fig.3.4) can be expected to to be more or less isodiametric in shape, with or without intercellular spaces between them, and to have thin cellulose walls; pits, if present, are always of the simple type. Parenchyma, as its Greek derivation (literally 'poured beside') indicates, forms the basic packing tissue in which more specialized tissues appear to be embedded. Besides its packing function, parenchyma may also be concerned with photosynthesis and with the storage of stareh and other substances. In relation to these two functions parenchyma cells vary considerably both in shape and living contents. Thus the parenchyma cells of the photosynthetic tissue of a leaf are rich in chloroplasts and may be considerably elongated or lobed. While those found in food-storing structures such as a bean seed (Phaseolus) or a sweet potato (Ipomoea batatas) are packed with starch grains. In addition to forming a simple tissue, parenchyma cells are a regular component of the two

Contd...P/6.

complex tissues, xylem and phloem, where they commonly serve for the storage of various substances, particularly starch.

Collenchyma :

This is a living tissue which has many of the characteristics of parenchyma, and may indeed be interpreted as a form of parenchyma structurally specialized as a supporting tissue in young organs. When collenchyma and parenchyma lie next to each other they frequently merge into one another through transitional types. The resemblance to parenchyma is also indicated by the common occurrence of chloroplasts in collenchyma, and by the ability of this tissue to resume meristematic activity. Collenchyma occurs immediately beneath or near the surface of young stems and petioles, and along the main veins of foliage leaves; it is rarely found in roots. The cells of collenchyma (Fig. 3.5) are elongated in the direction of the long axis of the organ in which they occur, and are characterized by the presence of thick, non-lignified, primary walls. The wall thickening, however, is not uniformly deposited around the inside of the cell but is thickest in the corners of the cell (angular collenchyma), although the two tangential walls may also be thickened (lamellar collenchyma). In longitudinal section therefore collenchyma shows thin and thick portions depending on the plane of the cut (Fig.3.5b).

The thick cellulose walls of collenchyma combine considerable tensile strength with plasticity. Hence collenchyma is particularly sutiable for the support of actively growing organs because its cells can extend to keep pace with the elongation of the organ and yet retain their strength.

Sclerenchyma :

Whereas collenchyma is the main supporting tissue of actively growing organs, sclerenchyma performs a similar function in mature plant parts. Sclerenchyma cells have thick secondary walls, usually lignified, and their protoplasts are dead, or at any rate inactive, at maturity.

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Sclerenchyma is a very variable tissue but two broad categories of it can be recognized, fibres and sclereids; in general fibres are very much longer than sclereids.

Fibres Fibres are long narrow cells with tapering pointed ends (Fig.3.6). They are usually massed together in long strands, their tapering ends overlapping one another and fitting tightly together. While fibres are young and actively growing, their end walls tend to slide over one another to produce the pointed state of the mature fibre. The increase in length of a fibre by the intrusion of its growing tips between the walls of neighbouring cells is called intrusive growth. After a fibre has ceased to elongate, its walls continue to thicken until at maturity the cell cavity is very much reduced, and sometimes almost obliterated. Fibres occur in most parts of the plant body and, according to their position, they may be classified into two types: xylary fibres which occur in the complex tissue xylem, and extraxylary fibres which occur in any tissue other than xylem. Xylary fibres have reduced bordered pits, the inner apertures being slit-like and those of a pit-pair often crossed with each other. Extraxylary fibres have very narrow, simple pits. Although the thick secondary walls of fibres are usually lignified, some fibres have unmodified cellulose walls (e.g. those of the flax plant (Linum usitatissimum), from which linen is made). Xylary fibres are a major component of wood, and on account of their heavily lignified walls, are responsible for the hardness and rigidity of this material. Extraxylary fibres, some lignified and others not, are common sources of rope, sacking and clothing textiles.

Sclereids :-

These are very variable in shape, ranging from approximately isodiametric to very irregular, and they may even be branched (Fig. 3.6 f-h). They have very thick, lignified walls, often showing concentric layering, which are pierced by numerous simple pits. [illegible]

[illegible]

[illegible]

[illegible] small [illegible]

[illegible] the flesh

[illegible] or as [illegible] in

[illegible]

Frequently the pits become branched as a result of the fusion of two or more pit cavities during the increase in thickness of the secondary wall, Sclereids may occur either singly or as small clusters among other cells in, for example, the gritty specks in the flesh of a guava (Psidium guajava), or as continuous masses as in the hard shell of a coconut (Cocos nucifera).

Complex tissues :-

Although a complex tissue is composed of several different types of cells it merits being recognized as a single tissue because it has certain features peculiar to itself, occurs in definite positions in the plant body, and is associated with definite functions. There are two complex tissues, the xylem which is concerned primarily with the conduction of water and inorganic solutes, and the phloem which is concerned primarily with the conduction of food manufactured in the leaves. These two complex tissues always occur side by side, and together constitute the vascular or conducting system which extends throughout the plant.

Xylem :-

The xylem is composed of parenchyma and fibres, which have already been described, and tracheids and vessel members, which are characteristic of this tissue. These four elements occur in different proportions and combinations, and all four are not necessarily present in the xylem of any given plant.

Tracheids :-

Tracheids are more or less elongated cells, which are angular in cross-section and have oblique or tapering end walls (Fig. 3.7). Tracheids are dead at functional maturity when they consist only of lignified cell walls. All tracheids have secondary walls but these are deposited in various patterns which are related to the state of maturity of the region in which the tracheid is being formed

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(Fig. 3.8). In the tracheids formed nearest to the apical meristem the secondary walls are deposited as a series of horizontal rings (annular thickening) and in those further back as one or more spirals (helical thickening). These are succeeded by tracheids in which the secondary wall forms a net-like pattern (reticulate thickening) over the primary wall, and finally by tracheids in which the secondary wall is continuous except for clongate or circular pits (pitted walls). These different patterns of secondary wall thickening form a series in which progressively more of the primary wall is covered, but the different types often merge and not all types are necessarily represented in any given plant. All four types of thickening give support to the tracheids in which they occur, so that the central cavity or lumen is kept open despite pressure from neighbouring cells. It is interesting to note, however, that tracheids with annular and helical thickenings are formed while the surrounding tissues are still elongating, and they continue to be stretched even after they are structurally mature. The occurrence of stretching is shown by the fact that, when several helical tracheids are present end to end in a longitudinal series within the region of elongation, the spirals of the older tracheids are more drawn out than those of the younger ones. Tracheids with more extensive, and therefore more rigid, wall thickening are not readily stretched, and such tracheids differentiate in plant organs which have stopped elongating.

Water moving through tracheids passes from one cell into the next through the areas, whether extensive or restricted to pits, where the primary wall only is present. Tracheids perform the double function of a supporting and water-conducting element, and in such vascular plants as ferns and conifers, which are more primitive than angiosperms, they are the only cell type present in the xylem apart from parenchyma. During the course of evolution the tracheid diverged along two routes, one in the direction of greater mechanical efficiency which resulted in the xylary fibre

Contd...P/10.

(already described), and the other in the direction of greater water-conducting efficiency which resulted in the vessel member. Tracheids, however, were not eliminated when fibres and vessel members were evolved.

Vessel members These are cylindrical cells, non-living when mature, which are joined end to end to form multicellular water-conducting tubes, called vessels. The end walls (and sometimes also the side walls) of vessel members become perforated by one or more holes through which water can pass freely from cell to cell (Fig.3.7d-f). Hence, instead of a tier of superimposed cells, there is formed a tube rather like a drainpipe in structure, which is the vessel. Vessel members are commonly shorter and much wider than tracheids, but they develop lignified secondary walls in a similar way and show the same patterns of wall thickening (Annular, helical, etc.) as tracheids.

Vessel members begin life as separate cells with a continuous primary wall, but at a certain stage in development the middle lamella swells in the areas which are to become perforated (Fig.3.9). Subsequently a secondary wall develops, but this is not deposited on the areas (e.g. primordial pits) which persist as thin primary wall or on the areas of future perforation. As the vessel members mature, the primary walls and middle lamella in the perforation areas break down, so leaving open holes. The perforated areas (called perforation plates) may be simple, with one large perforation, or multiperforate, with more than one perforation (Fig.3.7d-f,h,i). In a mature vessel with simple perforation plates the previous position of each cross-wall is often indicated by the presence of a distinct rim of thickening around the lumen of the vessel. Vessels are not of indefinite length, although they frequently extend for several metres. Where a vessel ends, the terminal vessel member is perforated at its proximal end but not at its distal end. Therefore the passage of water from vessel to vessel takes place through the areas of primary wall (such as pits) in a manner similar to conduction in tracheids.

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In transverse section vessels can usually be recognized by their large, almost circular outlines. Whereas the widest tracheids scarcely exceed 100 um in diameter and most are much narrower, vessels are commonly 300 um across and some, especially those of climbers, reach a width of 700 um. The wide diameter of vessels, coupled with the perforations in their end walls, enables water to be conducted more efficiently in these elements than in tracheids.

PHLOEM

The characteristic components of the phloem are sieve-tube members and companion cells, but parenchyma and, in some cases, fibres also occur.

Sieve-tube members These are elongated cells joined end to end into sieve tubes (Fig.3.10) in a manner analogous to the joining of vessel members to form vessels in the xylem. Unlike vessel members, however, the cell walls of sieve-tube members are not lignified and are classed as primary walls, even though they are usually thickened. Also unlike vessel members, the cross-walls of sieve-tube members, although much modified, do not break down completely. Instead, the cross-walls become perforated by open pores through which the protoplasts of two superimposed members are continuous. These connecting strands of cytoplasm are comparable with plasmodesmata but very much wider. The perforated cross-walls are called sieve plates because in surface view they resemble sieves (Fig.3.11). It is from the presence of sieve plates that this characteristic cell type derives its name. Usually the sieve plate covers the entire cross-wall, but sometimes it is broken up into several perforated sieve areas, thus forming a compound sieve plate.

In the sieve plate each cytoplasmic strand is enclosed in a cylinder of a special kind of carbohydrate called callose. -Aniline blue The thickness of this callose cylinder obviously affects the size of the sieve pore. When the plant is dormant thick callose pads completely block the pores, but at the onset of

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the growing season much of the callose is removed and new connecting strands of cytoplasm appear. If the plant is injured callose is also deposited, often within a few seconds of the injury, thus blocking the pores and minimizing loss of food material. One consequence of this instantaneous reaction is that in most microscopic sections the pores are completely blocked. This is the result of the treatment of the tissue for examination, and does not represent the situat in an actively growing plant.

Sieve-tube members are living cells and only function as long as they are alive. A remarkable characteristic of sieve-tube members is that during development the nucleus that was originally present breaks down and completely disappears at maturity. They are the only known example of plant cells which are living but lack a nucleus. Investigatio with the EM has indicated that sieve-tube members are also remarkable in possessing very few mitochondria and almost no other cyto~~plasmie-org~~anelles. Furthermore, the normal distincti between vacuole and cytoplasm seems to be lacking, the main volume of the cell being occupied by a fibrillar protein, called P-Protein, whose exact structure and function is currently the subject of active debate among plant physiologists. The proteinaceous material readily takes up staining reagents, and in stained longitudinal sections usually appears attached to the sieve plates as so-called slime plugs. The presence of slime plugs provides one of the most useful criteria for identifying the position of phleom in longitudinal sections (Fig.3.10a).

Companion cells Each sieve-tube member is closely associated with one or more slender parenchyma cells, called companion cells, which have dense cytoplasm and conspicuous nuclei. Companion cells arise by an unequal longitudinal division of the same mother cell as the sieve-tube member (Fig.3.12). After being cut off from the sieve-tube member the companion cell does not necessarily remain as a single cell but may divide horizontally into a vertical file of

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companion cells. Companion cells always lie alongside a sieve-tube member, and the cytoplasm of the two partners is in direct contact through plasmodesmata in the pit membranes on the side walls. The two types of cells form a single physiological unit because the companion cells die when their associated sieve-tube members cease to function. The nature of the interdependence is not known, but it can be assumed that the sieve_tube member, which has no nucleus of its own, depends on the nucleus of the companion ~~cell to su~~pport those activities for which a nucleus is normally essential.

TISSUE SYSTEMS

Just as individual cells are arranged into different types of tissue, so individual tissues in theirturn are arranged in definite patterns throughout the plant body. Thus the tissues concerned with the conduction of water and food form a continuous system extending throughout the entire plant. These so-called vascular tissues connect places of water intake and food synthesis with regions where water and food are used for such functions as growth or storage. The non-vascular tissues are similarly continuous, which again indicates the interdependence of leaves, stems and roots. The individual tissues (parenchyma, selerenchyma, xylem, phloem, etc.) are thus organized into larger units called tissue systems. In this book the main tissues of the plant are classified on the basis of their spatial continuity into three tissue systems, the dermal, the vascular and the ground (or fundamental) systems.

The dermal system, as its name denotes, forms the outer covering of the plant. It includes both the epidermis (i.e. the continuous layer which is formed over the surface of the primary tissues of the plant body) and also the periderm (i.e. the protective tissue which replaces the epidermis near the surface of stems and roots which undergo secondary thickening). Dermal tissues have special characteristics, such as the impregnation of their walls with cutin or suberin, which are related to their superficial position. The vascular system is concerned with the conduction of water and food throughout the plant, and contains two kinds of conducting tissues, the xylem

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(conducting water) and the phloem (conducting food). Beca of the presence of fibres in them, the vascular tissues, particularly the xylem, are also concerned with support. Th ground system includes the tissues that can be regarded as forming the ground substance in which the vascular tissues are embedded. The main ground tissues are the three simple tissues, parenchyma, collenchyma and selerenchyma.

Within the plant body the three tissue systems are distributed in characteristic patterns depending on the orga in which they occur, on the taxonomic group to which the plant belongs, or on both (Fig.3.13). Since the dermal syste is always superficial, the principal differences in pattern depend on the relative distribution of the vascular and ground systems. In a dicotyledonous root, for example, the vascular tissue usually forms a central rod embedded in the ground tissue (cortex). In a dicotyledonous stem, however, the vascular tissue typically forms a hollow, perforated cylinder with some ground tissue (pith) enclosed within the vascular cylinder and some located between the vascular cylinder and the dermal tissue. The latter region of ground tissue is called cortex because, like the ground tissue in the root, it occurs outside the vascular tissue. This method of interpreting the anatomical pattern of an organ is not only descriptively useful, but also reflects the basic unity underlying the organization of the plant as a whole.

Whatever the arrangement of tissues in a mature organ may be, the pattern is established immediately behind the apical meristem from which it is derived. In this subapical region, where the cells are still actively dividing. It is thus possible to recognize three types of meristematic tissue. The protoderm, ground meristem and procambitum. These are sometimes called the three primary meristems because they are the precursors of the dermal,ground and vascular tissue syste respectively. Although leaves originate on the flanks of the shoot apex, these three types of meristematic tissue are also present in a developing leaf.

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Table 8.1 Characteristic features, funtions and distribution of plant tissues.*

Tissue	Living or dead	Wall material	Cell shape	Main functions	Distribution
Parenchyma	Living	Cellulose, pectins and hemicelluloses	Usually isodiametric sometimes elongated	Packing tissue support in herbaceous plants. Metabolically active. Intercellular air spaces allow gaseous exchange. Food storage. Transport of materials through cells or cell walls.	Cortex Pith, Medualiary rays and packing tissue in xylem and phloem
Modified Parenchyma					
a) epidermis	Living	Cellulose, pectins and hemicelluloses and covering of cutin	Elongated and flattened	Protection from desiccation and infection. Hairs and glands may have additional functions.	Single layer of cells covering entire primary plant body.
b) mesophyll	Living	Cellulose, pectins and hemicelluloses	Isodiametric, irregular or column-shaped depending on location	Photosynthesis (contains chloroplasts). Storage of starch.	Between the upper and lower epidermis of leaves
c) endodermis	Living	Cellulose, pectins and hemicelluloses, and deposits of suberin	As epidermis	Selective barrier to movement of water and mineral salts (between cortex and xylem) in roots, Starch sheath with possible role in geotropic response in stems.	Around vascular tissue (innermost layer of cortex)
d) pericycle	Living	Cellulose, pectins and hemicelluloses	As parenchyma	In roots it retains meristematic activity producing lateral roots and contributing to secondary growth if this occurs	In roots between central vascular tissue and endodermis

Contd...P/16.

Collenchyma	[illegible]	[illegible] hemicelluloses	Elongated and polygonal with tapering ends	Support (a mechanical function)	Outer regions of cortex e.g. angles of stems, midrib of leaves
Sclerenchyma					
a) Fibres	Dead	Mainly lignin, Cellulose, pectins and hemicelluloses also present.	Elongated and polygonal with tapering interlocking ends	Support (purely mechanical)	Outer regions of cortex, pericycle of stems, xylem and phloem.
b) sclereids	Dead	As fibres	Roughly isodiametric though variations occur	Support or mechanical protection	Cortex, pith, phloem, shells and stones of fruits, seed coats.
Xylem	Mixture of living and dead cells. Xylem also contains fibres and parenchyma which are as previously described.				
tracheids and vessels	Dead	Mainly lignin, cellulose, pectins and hemicelluloses also present.	Elongated and tubular	Translocation of water and mineral salts. Support	Vascular system.
Phloem	Mixture of living and dead cells. Phloem also contains fibres and sclereids which are as previously described.				
a) sieve tubes	Living	Cellulose, pectins and hemicelluloses	Elongated and tubular	Translocation of organic solutes (food)	Vascular system
b) Companion cells	Living	Cellulose, pectins and hemicelluloses	Elongated and narrow	Work in association with sieve tubes	Vascular system

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* Tissues associated with secondary growth, such as wood and cork, are described in chapter 21.

PLANT - TISSUES
PRACTICAL

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APPENDIX - I

The following brief notes on certain phases of microtechnique are given here to facilitate the use of this book by the teacher and student. For full information on the various procedures used in preparing tissue for microscopic study, reference should be made to the publications of Chamberlain (1932), Rawlins (1933), Johansen (1940), and Sass (1940), [illegible] General References.

FREE-HAND SECTIONS

In many of the exercises in this book, directions are given for the study of sections cut by hand from living stems, leaves or other plant structures. To prepare such material requires only simple technique and in addition provides a realistic picture of cells and tissues that should precede the examination of microtomed and permanently stained preparations. In the laboratory the student can acquire the necessary skill with a sectioning-razor to enable him to explore the structure of such tissues as the epidermis, parenchyma, collenchyma, and the phloem and xylem of vascular bundles. Best results are secured by enclosing small portions of the material between the split halves of pieces of elderberry pith or of carrot root. Often it is necessary to make a groove in the inner surface of one of the pith halves or strips of carrot to accommodate such bulky objects as stems, petioles, etc. In sectioning, hold the object enclosed between the pieces of pith at as constant an angle as possible and transfer each section to water; the slices of pith can be removed by the use of a brush. Sections cut by hand should be carefully mounted on a clean slide either in distilled water or in the various reagents designated and the cover-glass lowered gently into place. For more resistant cells, such as sclereids or fibers and for the critical study of the sieve-plates in phloem elements, the use of the carbon dioxide freezing microtome is highly desirable. With the aid of this

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instrument a large number of thin sections may be prepared by the instructor in advance of class use (for a description of the technique of sectioning with the freezing microtome, cf. Sass, 1940, pp. 97-98). The student must learn to check free-hand preparations at frequent intervals so that the sections are not allowed to dry out. Cells immersed in fluid are not only easier to study from an optical point of view, but they also retain a more or less normal structure over a relatively long period of observation. Sections of hairy objects, such as many leaves or stems, are often difficult to mount in water without the formation of numerous air-bubbles. This difficulty may be removed by mounting each section in a weak solution of alcohol. This acts as a killing reagent for the protoplasm, but it does make possible the accurate study of the shape, arrangement, and character of the walls of cells.

PREPARED SLIDES

The use of permanent slides is essential in the study of many of the topics outlined in this book. This is particularly true for the work to be done under Exercises III, X, XII, XIII, XIV, and XV. Suitable preparations as a basis for class study are obtainable from commercial supply houses or may be prepared for the student directly. With reference to the latter possibility, detailed suggestions for the collection, fixation, sectioning, and staining of tissues and organs are presented systematically in the recent manuals on microtechnique by Johansen (1940) and Sass (1940).

MACERATED TISSUE

One of the most important skills that the student must develop in laboratory practice is the ability to visualize cells as three-dimensional bodies. This is often extremely difficult on the basis of the examination of sections that tend to create a two-dimensional concept. Furthermore, many definitive features of cells, particularly the structure and arrangement of pits and the varied secondary wall thickenings in tracheary

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elements the character of perferations in vessel elements and the form of ramified selereids, can best be studied in isolated cells. For these reasons, a study of macerated tissue is recommended for many topics in this book and is especially desirable in connection with Exercises II, VIII, IX, and X. The maceration of plant tissue is most effectively accomplished by the use of certain reagents that dissolve the intercellular substance and thus cause the separation of a piece of tissue into its component cells. Jeffrey's method is usually satisfactory, especially for hard lignified structures. Small pieces of the material, no thicker than a match, are placed in a glass vial containing a mixture of equal parts of 10% chromic acid and 10% nitric acid. The vial is then corked and placed in an electric oven at a temperature of 30°-40° C. until the material becomes soft or "mushy" in texture. Hard material, such as wood and the shells of nuts, may require several days in the oven, during which time it is advisable to change the macerating fluid once or twice. Boiling small slivers of wood before placing them in the acids drives out the air and accelerates the maceration process. It is advisable to stop the action of the macerating fluid before a complete separation of all cells has occurred. Small pieces of "mushy" tissue, placed in water on a slide, can be teased apart with needles; such a procedure yields very instructive preparations of connected cell groups as well as isolated elements. The macerated tissue is carefully washed in distilled water to remove as much of the acid as possible and can them be transferred to 50% alcohol for future study. Often effective results may be secured by staining the isolated cells in safranin. Permanent preparations of macerated tissue are easily made by placing small quantities of cells in water on a slide, evaporating the excess water on an electric hot-plate and mounting in glycerine jelly. Circular cover-glasses should be used, the edges of which can be sealed with some type of cement which prevents drying out and the entrance of air.

For some tissues, for example, the cortical and pith parenchyma of herbaceous stems and the mesophyll of leaves, the following more gentle maceration technique should be employed. Place small portions of the tissues in acid-alcohol (3 parts of 70% ethyl alcohol; 1 part concentrated HCI) and thoroughly

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remove the air by means of an aspirator. Add fresh acid-alcoh and allow this to act on the tissue for 24 hours. After thoro washing in water, transfer the tissue to a 0.5% aqueous soluti of ammonium oxalate. Within a few days (or sooner depending u the material) the parenchyma or collenchyma tissues can readil be dissociated, by gentle teasing with needles, into their component cells. This method is highly recommended for isolati the idioblastic ramified sclereids of such plants as Camellia, Osmanthus, etc., from neighbouring tissue elements.

Stain Preparation

1. Safranin : Dissolve 1 gm of dye in 50 per cent alcohol.
2. Fast green : Dissolve 1 gm of dye in 90 per cent alcohol.

Mounting

All the stained thin sections are transferred to drop of Canada Balsam (mounting media) taken on the slide. Covered with cover slip a permanent stained slide lasts longer.

IX Maceration

Sections of the plant parts do not depict the actual nature of the constituent cells. The cells can be studied by dissociation method called maceration. The materials are treated with chemicals which dissolve the middle lamella of the cell wall. This also dissociates the cells from one another. The tissues like xylem and phloem depict the actual nature of the constituent cells.

Method

Cut the material into small pieces and treat it for 24 hours with acid alcohol solution (HCI, alcohol). Wash the materials and transfer to 0.5% ammonium oxalate solution and boil Mount the tissue on the slide in drop of above solution and stud

Macerating Fluid

1. Ammonium Oxalate Fluid

Hydrochloric acid	30 cc
Alcohol 95 per cent	100 cc
Ammonium oxalate	0.5 per cent

Contd...P/

2. Schule's Fluid

Potassium chlorate	1 gm
Nitric acid	50 cc

The materials are heated in the mixture for sometime, washed and mounted in glycerine.

CLEARING TECHNIQUE

It is extremely laborious and in many cases unnecessary to attempt to reconstruct the gross anatomy of the vascular system of a flower or the venation of a leaf by means of serial sections. The following procedure yields very instructive preparations and is applicable to herbarium specimens as well as to fresh material.

1. Leaves. Small leaves can be cleared in toto. Large laminae, however, should be subdivided into strips (about ¾ inch in width) extending from the mid-rib to the margin. The leaf material of herbarium specimens should first be soaked in hot water until it sinks; with fresh leaves, the chlorophyll should be extracted by means of hot alcohol. Following these preliminary treatments, the material is then transferred to vials containing a 5-10% aqueous solution of NaOH. With delicate objects, the removal of the cell contents may be achieved without heat. But for thick, coriaceous leaves, the vials should be placed in an electric oven. Frequent changes of the reagent are desirable until the leaf material appears devoid of discoloration. After washing thoroughly in distilled water, the material should be examined (without a cover-glass) under low magnification; in many cases the stomata, veinlets, and other histological features (e.g., idioblastic sclereids) can now readily be studied. Cleared material at this stage can be stored for future examination in 50% alcohol. Very often, however, even after several days treatment with NaOH, the material may still be opaque. In such instances, dehydration in successive grades of alcohol followed by clearing with xylene or toluene are necessary. Syracuse watchglasses are convenient receptacles and the following series is recommended: 50% alcohol, 95% alcohol, 100% alcohol (2 changes), 100% alcohol-xylene, pure xylene. Use a camel-hair's brush or

forceps in transferring the material through this series, draining off the excess fluid on filter paper at each change. Because of the large volume of tissue represented, dehydratio must be very thorough or a milky turbidity will form when the final transfer to xylene occurs. To avoid this, allow the material to remain in each of the grades of alcohol for at le 10-15 minutes. After several pieces of leaves have passed through the entire series, the reagents should be discarded an fresh alcohol and xylene added to the watch-glasses. To make permanent mounts, transfer a piece of material to a clean sli flooded with xylene, add a generous amount of balsam and carefully lower a cover-slip into place. The slides should then be placed on an electric-slide warmer for a week or longer until thoroughly dry. Various types of stains (e.g. safranin, Delafield's hematoxylin) often increase the usefulness of cleared leaf-material. These stains may be introduced at various points in the alcohol-xylene series.

2. Flowers. The procedure outlined above is also applicable to the study of the vasculation of the floral axis and its appendages. In making permanent mounts of cleared flowers it is often desirable to use "depression slides"; these permit the inclusion under the cover-slip of relatively thick objects.

SPECIAL REAGENTS

1. Phloroglucinol and hydrochloric acid. The addition of these reagents produces a red color in the walls of sclereid lignified fibres, and tracheary elements (of Exercises VIII, IX and X). The stain it not permanent but nevertheless is extremely useful in demarcating the thick walls of certain types of cells. A saturated solution of phloroglucinol should be prepared in 18% HCL. Mount the section or tissue fragment directly in a drop of this reagent on the slide and add a cover-slip. Great care should be taken to carry out this procedure some distance away from the microscope.

2. Potassium Iodide (IKL) and sulphuric acid. This is a specific test for cellulose. Mount the sections in the potassium iodide (1g. iodine and 3g potassium iodide in 300cc.

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of distilled water, according to Rawlins, 1933) and add a cover-slip. The introduction of a drop of 65% sulphuric acid will cause cellulose walls to turn blue in color.

3. **Anilin blue.** This is a specific stain for callus depositions on sieve plates and is essential for the procedure outlined in Exercise XI. Sections should be immersed for a short time in a 1% aqueous solution, and then transferred, after gentle washing, to a drop of water. The callus on the sieve plates is stained blue. Dr. A.S. Crafts has suggested to the writer the following improvement: Place the sections in IKI, wash in water, stain in anilin blue for about five minutes, then wash briefly again with IKI and mount for study in tap water or glycerine.

4. **Neutral red.** This vital stain is very useful as a general stain for the primary walls of living cells, and is recommended for use in Exercises V, VI, and VII. Mount the sections directly in a 1% aqueous solution.

5. **Sudan IV.** This reagent is specific for the cuticle and for cutinized and suberized cell walls. Place the sections in a drop of alcoholic solution of Sudan IV (5g. in 100cc. of 80% alcohol), add a cover-glass, and examine under the microscope. The cuticle, as well as waxy materials present in walls, are stained red. This reagent is very desirable for use with Exercise V.

* * *

The Tissue System

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Introduction

The cells in the body of the multicellular animals vary in structure and function. It is seen that one variety of cell performs one kind of work and constitutes one type of tissue.

Definition

So a tissue may be defined as an aggregate of same types of cells i.e. similar in morphology, physiology and embryonic origin, combined by subserving the same general function independently and united by varying amounts of intercellular substance (e.g., blood, bones, cartilage, muscle, nervous tissues etc.).

Origin

The first few cells undergo repeated miotic division. The first evidence of differentiation is discernible in this cellular mass, where distinct layers, known as the ectoderm, the mesoderm and the endoderm. Cells of each of these layers reorganise themselves to form tissue system.

There are four primary types of tissues in the body:

i) Epithelial - which covers surfaces, lines cavities and forms tubes.
ii) Connective - which forms supporting and binding structures.
iii) Muscular - which comprises the contractile elements.
iv) Nervous - which makes up the regulating and conducting structures.

The Principal differences among the various types of tissues arise from the types of cells of which they are composed, the nature and amount of intercellular material present, and the functions they perform.

1) Epithelial Tissue

Forms the covering of the outer surfaces of the body and of most internal organs, lines the digestive and respiratory tracts, the serous cavities, blood vessels, excretory ducts and reproductive ducts and organs. It comprises the secreting portions and ducts of glands and the sensory portions of sense organs.

Structural characteristics of epithelial tissue

1) Epithelial cells are arranged in a continuous sheet, usually of one layer.

2) They are packed together closely with little intercellular substance.

3) Blood vessels are absent, but nerve endings are usually abundant.

4) Epithelial cells lie on a basement membrane, a t layer of intercellular substance, permeated with reticular fibers.

5) The cementing substance, here, is a mico-protein containing hyaluronic acid and calcium. salts.

Types of Epithelium

Differentiated on the basis of

(a) Shape of the cells

Squamous Cuboidal Columnar Transitional

and (b) Their arrangement in the epithelial sheet

Simple Strati

According to the origin and association with other tissue it may be classified as:

Epithelium	Mesothelium	Endotheli
Form membranes associated with a thin connective tissue layer	Epithelium when derived from mesoderm e.g. peritoneum	Lines the surface o blood vess and heart

a) Based on the shape

Squamous	Cuboidal	Columnar	Transitio
Consists of cells which are thin and flat, with regular or irregular outlines	Consists of cube-shaped cells or cells in the form of truncated pyramids	Cells are long, cylindrical sometimes forming tall, irregular prisms.	Resembles s tified epit in that it c sists of se layers of ce but its supe ficial surfa cells are l and rounded stread of b squamous in The no. of l varies with condition o organ, as i urinary bla

Function

1) Protection
2) Absorption
3) Secretion
4) Excretion

a) Squamous:

i) Simple (cells in a single layer) lining the body cavities and in Bowman's capsule of the kidney.

Mesothelium: that lining the serous cavities (pleural, peritoneal, pericardial)

Endothelium: that lining the blood and lymph vessels.

ii) Stratified: Squamous epithelium (cells in several layers) is found in epidermis ,cornea,oesophagus and vaigina.

b) Cuboidal:

i) Simple cuboidal: Certain glands and parts of the kidney tubules(thyroid), the ducts of glands, and the smaller bronchi.

ii) Stratified Cuboidal: Epidermisof certain tailed amphibians.

c) Columnar Epithelium: Consists of cells which are long and cylindrical, sometimes forming tall, irregular prisms.

Six Subtypes:

i) Simple : in the lining of the intestine.

ii) Simple ciliated: in the oviducts, uterus and nasal sinuses.

iii) Stratified: in the pharynx, on the epiglottis

iv) Stratified ciliated: in the larynx on the soft palate.

v) Pseudostratified: varying in height with nuclei at different levels, all cells touching basement membrane, in the parotid gland and male urethra

vi) Pseudostratified ciliated: in the trachea and Eustachian tube.

d) Transitional Epithelium

Consists of several layers of cells (like stratified epithelium) but its superficial surface cells are larger and rounded intead of being squamous in shape.

The number of layers varies with the condition of the organ as in urinary bladder.

Free surface of Epithelial cells

The free surface of epithelial cells may be modified in various ways. In some cases, the superficial protoplasm is involved; in others, a membrane-like secretion, or cuticle is formed.

Modification of superficial protoplasm

The borders of the epithelial structures may be either striated, ciliated or of the brush type.

The striated border is found in cells of columnar intestinal epithelium, usually in area where absorptive processes take place. It consists of fine striations lying perpendicular to the surface of the cells. The ciliated border is representative of respiratory epithelium cilia a thin, very numerous, hair-like projections of varied leng At the proximal end of each cilium is a small basal corpus Ciliary movement results from the quick bending of the cili in one direction and a subsequent straightening or recovery stroke. The action of each cilium follows that of the ad- joining one in a wave-like action which is repeated. In th manner mucus or particles in contact with the cilia are mov slowly along the ciliated surface. The brush border is fou in cells comprising of certain protions of kidney tubules. consists of fine, hair-like processes which are nonmotile. gives the appearance of a dense brush.

Other specialised borders include: Stereocilia, nonmotile projections in cells of the epididymis which are thought to aid in eliminating the secretion of the cells, a nonmotile hairs in hair cells of the utricle, saccule, a cochlea of the ear, serving as receptors for vibratory stim Mature spermatozoa possess a flagellum, a single hair-like proceeses which serves for locomotion

Structure of Cuticles

A cuticle is a layer of more or less solid substance which is secreted by and covers the free surface of an epi- thelial sheet. It is usually sharply delimited from the ce surface and capable of being separated from it.

Eg.- Enamel of teeth
Capsule of the lens of the eye

Binding together of Epithelial cells

The cells of an epithelial sheet are so closely bou to one another that it usually requires considerable mecha force to separate them. The factors operating to bind them together are:

1. Interstitial substance acting as an intercellular cement.
2. Desmosomes: Condensations of cytoplasm at points contact between epithelial cells.
3. Terminal bars, rod-like thickenings of the plasma membranes which cement together adjacent cells.

Glands

Because they arise from an epithelial surface, glands are included in this discussion of the structure of epithelial tissues - sweat glads, sebaceous glands, ceruminous glands, mammary glands.

Functionally a gland is a cell or group of cells that elaborates or manufactures a specific substance which is extruded from the cell into a surface or into the blood or lymph. The product thus formed does not become a part of the body tissues but is either absorbed and used by them or discharged from the body.

Structurally - a gland is a cell or an aggregation of secreting cells. Multicellular glands arise and develop by a process of invagination, the epithelium grows into the adjacent connective tissue and a simple tube-like or sac-like structure is formed. The cells in the closed portion of the sac assume a secretory function while those near the surface narrow to form an excretory duct. The more complex multicellular glands arise by repeated invaginations, the result is a complicated, branched organ.

Secretion

Secretion is the process whereby a cell obtains materials from the blood and lymph and transforms them into products which are passed from the cell.

Polarization:

Gland cells (and, for that matter most epithelial cells) are said to be polarized; that is to say their proximal or basal end (that nearest to the basement membrane) is different in structure from the distal or free end. The nucleus lies at the proximal end, whereas, in the active phase of secretion, the secretory products(granules, mucigen or other substances) fill the distal end, from which they are discharged.

Classification of Glands

Glands may be classified on the basis of

i) the presence or absence of excretory ducts.
ii) the nature of secretion.
iii) whether the secretion is a product of the cell or a part of the cell itself.
iv) their structure.

1) Presence of excretory ducts: Based on the presence or absence of ducts the glands can be

i) Exocrine or external secreting glands: With ducts and empty their products onto a free surface(eg. salivary gl

ii) Endocrine or internal secreting glands are ductless; th secretions are absorbed into blood or lymph(e.g. thyroi gland). Some glands, for example the pancreas, ovary or tes are double-functioning, serving as combined exocrine and en crine glands.

2) Nature of secretion: Depending upon the nature of secret glands can be

Mucous glands:	Secrete mucous, a viscid substance which principally contains mucin(e.g. globlet c of intestine, tracheal glands).
Serous glands:	secrete a clear, watery abuminous fl (parotid gland). salivary gl
Mixed glands :	Contain both mucous and serous secretory (submaxillary gland) Salivary gl

3) Secretion of a product or a part of the cell :

Merocrine gland	: The secretory product is the only par extruded (salivary gland)
Apocrine	: the apical end of the cell containing accumulated products is broken off an extruded; the remaining protion of th cell is left intact, the cell reforms and the process is repeated(mammary g
Eccrine	: Similar to apocrine by cytoplam is broken down before discharge.
Holocrine	: The entire cell, along with its conta secretory product, is extruded, the c is replaced by a new cell (sebaceous oil gland)

4. Classification of glands by structure:

i) Unicellular: Found on free surfaces, they secrete muco e.g. goblet cells of intestine.

ii) Multicellular

- Tubular
- Albelar (acinous)
- Tubuloalveolar

Tubular glands: have their secreting portion in the form of blind, narrow tube.

Simple

a) Simple straight (e.g. in large intestine)
b) Simple coiled(Sweat glands)
c) Simple branched(gastric glands)

Compound

With a large number tubes branching rep (in kidney, testis mammary glands of laying in mammal).

Alveolar glands

Have a flask-shaped secreting portion called an alveolus or acinus. They are

i) Simple (Mucous and poison glands of frogs and toads)
ii) Simple branched (sebaceous glands)
iii) Compound (Mammary glands)

The compound tubuloalveolar gland has a secretory portion that consists of irregularly branched tubules and saccular outgrowths (e.g. majority of endocrineglands(~~salivary~~, pancreas).

CONNECTIVE TISSUE

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1. Definition:

Most widely distributed occurs almost in every part of the body, constitute some part of almost every organ.

2. Function

Forms the supporting and connecting structures of the body.

3. Origin

With one or two exceptions, connective tissues arise from the mesoderm, more specifically they arise from the mesenchyme of this layer.

4. Difference with epithelial tissue

i) The cells are not aggregated.
ii) A considerable amount of intercellular substance present. which is always product of the cells of the tissue.
iii) This substance is called ground substance or matrix.
iv) As the development of embryo proceeds, the nature of intercellular substance changes.
v) Fibers begin to appear, first the collagenous, then the elastic.

General characteristics of connective Tissue -

I) The cells of connective tissue are relatively few in number, the bulk of the tissue consisting of intercellular material or matrix.
ii) They are highly vascular.
iii) Rarely do they occur on free surfaces.

Classification of connective tissue

1) Connective tissues are classified on the basis of their intercellular substance.
   i) Connective tissue proper: inter-cellular substance is of a fibrous nature.
   ii) Dense connective tissue, intercellular substance i.e. rigid or semirigid (cartilage, bone)

Connective tissue proper:

Four types

Mucous Fibrous Reticular Adipose

Mucous connective Tissue

1. Also known as Wharton's jelly,
2. It consists of branching cells, irregularly arranged which sometimes anastomose.
3. Jelly-like matrix present and contain mucin.
4. A few bundle of collagenous fiber may be present.
5. No elastic fibers.
6. Wharton's jelly is found only in the umbelical cord of the embryo.

Fibrous connective tissue

1. Composed of cells embedded in a matrix that is made up fibers and a semifluid substance.
2. Fibrous connective tissue is further subdivided into

- a) Areolar
- b) White fibrous (collagenous)
- c) Yellow fibr (elastic)

a) Areolar or loose connective tissue

1) It consists of an interlacing net work of fibers in a semifluid matrix throughout which cells are scattered. forms the interstitial tissues of mos
2) This kind of tissue/organs, surrounds blood vesse and nerves, and constitute most of the subcutaneo tissue and the deep fascia

b) The white fibers

1) Occur in bundles and if not stretched, are usuall wavy.
2) The fibers themselves do not branch, but a bundl of them may do so.
3) They are nonelastic yet flexible.
4) They swell in weak acids or alkalies and are digested by acid pepsin but resist alkaline trypsi
5) The collagen they contain yields gelatin upon boi

c) The yellow fibers

1) Are single fibers, usually straight but containin elastin, are highly elastic.
2) They may branch and anastomose
3) Resist boiling water, acids and alkalies and are digested slowly by both pepsin and trypsin.

Histology of Aerolar tissue

The cells of areolar tissue are :

1. Fibroblasts: The cells of areolar tissue are numerous, irregularly shaped phagocytic, thought to give rise to fibers.

2. Histiocytes: (fixed macrophages) irregular in shape with branching processes and small nuclei, stain deeply, normally quiescent but actively amoeboid and phagocytic during inflamatory process.

3. Plasma cells: Smaller than histiocytes, fewer in number, function unknown.

4. Mast cells: Occur in varying numbers, found especially along blood vessles. Origin and function are not definitely known.

   Produce heparin, an anticoagulant.

5. Miscellaneous cells: eosinophils, pigment cells, undifferentiated mesenchymal cells and lymphoid "Wandering" cells.

White fibrous connective tissue

1. Consists mainly of white fibres with few elastic fibres present.

Occurrence: It is found in the fibrous capsules of organs, tendons and aponeuroses.

Tendon: A tendon is a flat or cord-like band that serves to attach muscle to bone. Its collagenous fibres are arranged in parallel bundles.

Fibroblasts; the only cells present, are arranged in parallel rows between the bundles.

An aponeurosis is a flat sheet of connective tissue that attaches muscles to bones or other tissue; it is similar in structure to a tendon.

A ligament also is similar to a tendon in structure except that its fibres are less regularly arranged and some elastic fibres may be present.

Yellow fibrous or elastic connective tissue

It is made up of principally yellow elastic fibers with a few collagenous fibers. It is found in some ligaments and in the walls of large blood vessles, especially the aorta and the larger arteries.

- It is composed of scleroprotein called elastin, seen in areolar tissue, in ligaments between joints and vertebrae.

Reticular connective tissue

Reticular connective tissue consist of a syncytial network of cells with many argyrophilic intercellular fibers running in all directions and forming a reticulum. These fibers are so named because of their property of staining intensely with certain silver methods. This type~~s~~ of tissue forms the framework of lymphoid organs such as the spleen, lymph nodes and bone marrow. It is also found in certain endocrine glands in the walls of blood vesseles, and in digestive and respiratory passages underlying mucous membranes.

Adipose tissue

1) Adipose tissue is made up principally of cells that have the capacity for taking in fat and storing it

2) Mature fat cells contain a large droplet of neutral fat occupying the major portion of the cell.

3) The cytoplasm is reduced to a thin layer surroundi the droplet.

4) The nucleus is flattened and pushed to one side, giving the fat cell a "signet ring" appearance.

5) Groups of fat cells are separated by areolar tissu

6) Adipose tissue is found in the superficial fascia under the skin, around organs such as kidney, bladder, and heart, in mesenteries and the greater omentum, and as individ cells or in small groups in any loose connective tissue especially that along the blood vessels and nerves.

Functions

1) Serves as a reservoir of reserve food.
2) Protects the organs it ~~sa~~ surrounds against cold (or heat loss).
3) Protects against mechanical injury
4) Helps to support and hold organs in place.
5) Fills in the angular areas of the body.

Dense Connective Tissue:

1. This tissue includes a) Cartilage and b) bone osseous tissue

a) Cartilage: Forms foundation of endo~~skeleton~~ in all vertebrates and persists in this condition. throughout the life and in others partially at least in some vertebrates or wholly replaced by bone

There are three types of cartilage

i) Hyaline
ii) Fibrous
iii) Elastic

Hyaline Cartilage:

1) Consists of cells lying in cavities(lacunae) surrounded by a homogenous matrix or dense, semirigid intercellular substance.

2) The matrix contains collagenous fibers which, however, can be seen in ordinary preparations.

3) The cells called condrocytes, are usually single although they may occur in pairs or in groups of three or four.

4) Hyaline cartilage is flexible and slightly elastic.

5) It is covered by a membrane, the perichondrium, except over articular surfaces of the bone.

6. Hyaline cartilage is found in the articular cartilage~~s is found in the articular~~ covering the ends of bones at joints, in costal cartilages ~~bones at~~ between the ribs and sternum, and in the septum of the nose and cartilages of the larynx and trachea.

Fibrous Cartilage:

1) Consists of a matrix not quite so dense as that of hyaline cartilage and containing many collagenous fibers that are arranged more or less in rows.

2) It possesses great strength and ~~xx~~ flexibility.

3) The cells, avoid and enclosed in capsules, are relatively few in number and usually are widely separated.

4) This type of cartilage is found in intervertebral disks between the bodies of vertebrate and in the pubic symphysis.

Elastic Cartilage

1) It consists of a matrix having many elastic fibers ~~x~~ forming an interlacing net work.

2) Collagenous fibers are present but not readily seen.

3) The cells, usually single but sometimes in pairs, lie in cavities called lacunae.

4) This type of cartilage is flexible and elastic.

5) It is found in the cartilage of the external ear, the wall of the Eustachian tube, the epiglattis and certain layngeal cartilages.

Bone (Osseus tissue)

1) Bone is a rigid tissue consisting of bone cells embeded in matrix, that is, impregnated with calcium and phosphorous salts.

2) The structural unit is the Haversian system, which consists of cylinders of matrix and bone cells surrounding a cavity, the Haversian canal.

3) This canal contains blood vessels~~, lymphatics and nerves~~.

4) The matrix is arranged about each Haversian canal in concentric layers called lamellae.

5) Between the lamellae are lacunae, within which lie the bone cells (between osteocytes)

6) Many minute canals called canaliculi extend from each lacuna and penetrate the matrix in all directions, connecting the various lacunae.

PUMPING ACTION OF HEART

by Dr. J. K. Panda

The primary function of the cardio-vascular system is to provide an adequate supply to all cells of the body, of materials needed for their proper function and that carries away the waste products of their metabolism. It is a well-organised transport system of the body by which the blood being circulated within a closed system under different pressure gradients, created by the pumping mechanism where heart acts as the central pump. The volume of blood in our bodv is limited, but it has to perform unlimited amount of work continuously. This naturally leads to the conclusion that the same quantity of blood must be used over and over again. In other words, blood must circulate.

Blood gets reduced in the tissues and oxygenated in the lungs. Consequently, it has to pass alternately through lungs and tissues, doing opposite functions at these two places. Hence, circulatory system has been divided into two functionally opposite parts: (1) The systemic circulation (Greater circulation with high resistance circuit) - passing through the tissues. (2) The pulmonary circulation (Lesser circulation with low resistance circuit) - passing through the lungs. The two systems again meet in the heart.

ANATOMICAL CONSIDERATIONS OF HEART. The heart is roughly heart-shaped structure and rests obliquely in the thoracic cavity. The anterior surface of the heart faces the sternum, the posterior surface - the base of the cone faces the vertebral column and the inferior or diaphragmatic surface rests on the diaphragm. The heart has got four chambers two ventricles and two atria - both right and left. The two left chambers are separated from the two right ones, by a continuous partition, the atrial portion of which is called the interatrial septum (fibrous) while the ventricular part is known as the interventricular septum (upper one-fourth fibrous, lower three-fourths muscular). From the left ventricle arises the aorta, carrying

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oxygenated blood to the tissues. From the right ventricle, which is less muscular than the left, arises the pulmonary trunk, carrying reduced blood to the lungs. The right atrium receives all the venous blood from the body through three veins; the inferior and the superior venae cavae, and the coronary sinus. The left atrium receives all the oxygenated blood from the lungs through pulmonary veins.

Thus the four chambers of heart perform four different functions. The course of circulation is as follows. The left ventricle propels oxygenated blood to the tissues. Here, it gives up oxygen and becomes reduced. The reduced blood comes back to the heart through the veins and is received by the right atrium. From the right atrium it passes into the right ventricle, which then propels it into the lungs. Here, it becomes re-oxygenated, and is returned to the left atrium through the pulmonary veins. From here it enters the left ventricle and is pumped out into the greater circulation again. In this way circulation goes on. Two technical terms are used in connection with heart, e.g., systole and diastole. The term systole means contraction and diastole means relaxation.

VALVES OF THE HEART

There should not be any admixture between arterial and venous blood. In other words, circulation must be strictly must be strictly one way. This is done by the action of valves. There are four sets of valves in the heart. The right atrioventricular opening is guarded by tricuspid valve the left opening by the mitral or bicuspid valve. The openings of the aorta and pulmonary artery are guarded by semilunar valves (three cusps).

The cusps of tricuspid valve and mitral valve are triangular in shape and are attached at their bases to the margins of fibrous connective tissue encircling the atrioventricular orifices. These valves open when the blood passes from the atria to the ventricles. The apices of the valves are projected within the ventricles during flowing of blood into ventricles. But the apices are restricted to bulge within the atrium during ventricular contraction by the presence of chordae

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tendineae which attach the apical end of the valve and the papillary muscle in the ventricular wall at the other. The larger and stronger aortic semilunar valve, guards the orifice between the left ventricle and the systemic aorta, whereas the pulmonary (pulmonic) semilunar valve guards the opening between the right ventricle and the pulmonary trunk.

ACTION OF THE VALVES.

The atrioventricular (A.V.) valves open towards the ventricles and close towards the ventricles and close towards the atria. The semilunar (S.L.) valves open away from the ventricles and close towards the ventricles. So that when atria contract, atrioventricular valves open and blood passes into the ventricles. When ventricles contract, atrioventricular valves close, but semilunar valves open. This prevents regurgitation of blood into the atria but allows it to flow out of the ventricles. In this way circulation becomes one way.

The two ventricles contract simultaneously, as also the two atria. The same amount of blood passes out of the ventricles at the same time during systole. The same amount of blood enters the heart at the same time during diastole. Any discrepancy in the time or in the quantitative relations may ultimately cause heart failure.

SPECIAL JUNCTIONAL TISSUES OF THE HEART

Cardiac muscle consists essentially of certain specialised structures which are responsible for initiation and transmission of cardiac impulses at a higher rate than the rest of the muscle. Those specialised cardiac tissues operate such mechanism are collectively known as the junctional tissues of heart. They comprise the following structures : (1) Sino-atrial (S. A.) node. (2) Atrioventricular (A. V.) node. (3) Bundle of His (atrioventricular bundle). (4) The right and left branches of the bundle - ending in the (5) Purkinje fibres.

The sino-atrial and atrioventricular nodes and bundle of His are composed of specialised cardiac tissue and contain high amount of glycogen. These have got more sarcoplasm than

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the rest of the cardiac muscle fibres. Purkinje fibres also contain high amount of glycogen in their sarcoplasm. The atrial muscle fibre is connected with the ventricular muscle fibre only through the bundle of His because a fibrous tissue ring keeps the atrial muscle separated from the ventricular muscle. Damage of bundle of His causes dissociation of atrial and ventricular rhythm.

1. SINO-ATRIAL NODE. (Keith and Flack, 1907). It is situated in the right atrium at the junction of superior vena cava and the right auricular appendage. It extends downwards along the sulcus terminalis for about 2 cm (three-fourths of an inch). It is broader at the top and tapering below, and measures about 5 x 20 mm.

This nodal tissue possesses relatively few myofibrils and also it is claimed to consist of a dense network of small Purkinje fibres. Some investigators believe that the transmission of cardiac impulse from the S. A. node to the A. V. node is mostly facilitated by the presence of Purkinje fibres in the atria. No pathways of special fibres have yet been satisfactorily demonstrated until recently in the walls of the atria.

Goldman (1970) has described that there are three internodal atrial pathways originating from the S. A. node go to the A. V. nodal region (FIG. 1). These internodal tracts contain Purkinje type of fibres. The anterior internodal tract after coming out from the S. A. node curves round the superior vena cava and anterior wall of the right atrium. Here it bifurcates into two branches, one of which goes to the left atrium and other goes to the anterior superior region of the A. V. node. The middle internodal tract and posterior internodal tract after coming out from the S. A. node curve behind the superior vena cava and end in the superior margin and posterior margin of the A. V. node respectively. In between three internodal tracts there are interconnecting fibres which merge just above the A. V. node and also by-pass this node. In between the muscle cells, many nerve cells are found which act as relay stations for the vagus only. Excitor sympathetic fibres are also

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found here. Functions. It generates the normal cardiac impulse at the rate of 70 to 80 minute in the adult and acts as the pacemaker of heart and the rhythm originated from this region is generally designated as sinus rhythm.

2. ATRIOVENTRICULAR NODE (Tawara, 1906). It is situated in the right atrium at the posterior part of the interatrial septum close to the opening of the coronary sinus. It measures about 2 x 5 mm. The presence of the atrioventricular node was first identified by Kent in 1892 and afterwards His in 1893 described a band of modified muscle fibres to course from the atrium to the ventricle. Tawara (1906) described in details the presence of this specialised tissue in many species of animals. The cells of the A. V. node are cardiac muscle fibres but have a few myofibrils. It also consists of Purkinje fibres which form a dense network. The node is extended into the common-the atrioventricular bundle (bundle of His). Functions. (a) It receives the impulse originating from the S. A. node and transmits it to the ventricles through the bundle of His. (b) It acts as reserve pacemaker. The rhythm that is originated in the A. V. node is known as nodal rhythm. (c) It also initiates the cardiac impulse, but at a slower rate (40 to 60 per minute). In abnormal conditions, when the S. A. node fails, the A. V. node generates the impulse (nodal rhythm).

3. BUNDLE OF HIS. Course - The main trunk of this bundle is continuous with the A. V. node and passes upwards until it reaches the posterior margin of the membranous part of the interventricular septum and then forwards below it. It measures about 20 mm long.

4. BUNDLE BRANCH. Just above the muscular part of the septum, the bundle divides into right and left branches. The right bundle branch is longer than the left one. The left bundle branch bifurcates into superior and inferior divisions. It pierces the membranous septum, enters the left ventricle and passes along the muscular septum towards the apex. The left branch ends in the Purkinje systems of the ventricular subendocardial tissue (FIG. 2). The right branch passes down the right side of the septum. These branches remain just under the endocardium. They are finally distributed through the

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terminal arborisations of a special type of cardiac muscle fibres, known as the Purkinje fibres. Functions. (a) Conduction. Its normal function is to conduct the atrial impulse into the ventricles. (2) Rhythmicity - When the S. A. and A. V. nodes fail, the bundle can originate cardiac impulse. But the rate is very slow, about 36 per minute.

5. PURKINJE FIBRES. The Purkinje fibres which arise from the branches of the bundle of His, spread from the interventricular septum directly to the papillary muscle and then to the lateral walls of the ventricle ending ultimately within the subendocardial network. Purkinje (1845) first observed the presence of these fibres in the subendocardial tissue of the ungulate, heart. Purkinje fibres have got a larger diameter (50 to 70 p) than the ordinary cardiac muscle fibre (15 p). It also contains relatively more sarcoplasm with large amount of glycogen. Myofibrils in the fibre are present mostly in the periphery of cells and the central space is occupied by glycogen. Functions. Main function of these fibres is to conduct impulse quickly to every part of the ventricular muscle fibre. These fibres also can initiate impulse (30-35 per min.) in case of atrioventricular dissociation.

SPREAD OF CARDIAC IMPULSE

CONDUCTION OVER ATRIAL MUSCLE.

Cardiac impulse originated at the S. A. node is transmitted over both the atria like concentric waves. The spread of electrical impulse through the S. A node is very slow (0.05 m per sec.) but the same through the junctional tissues that connect the node to the atrial musculature or to the A. V. node is higher (1 m per sec.).

CONDUCTION OVER A. V. NODE.

There is also a considerable delay of 0.07 sec. to 0.1 sec. in transmission of impulse in the A. V. node before excitation spreads over the ventricle. This A. V. nodal delay allows the atrial systole to complete before the ventricle is excited. This delay is observed maximally at the junctional region between the atrium and atrioventricular node. The conduction velocity of impulse at this region is approximately 0.05 m per sec.

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CONDUCTION OVER BUNDLE OF HIS AND THE RIGHT AND LEFT BUNDLE BRANCHES.

Beyond the atrioventricular region, the impulse is transmitted along the bundle branch at a higher velocity (4-5 m per sec.). The impulse from the bundle of His passes quickly through the right and left bundle branches and ultimately reaches the Purkinje fibres and ventricular muscle fibres as well.

CONDUCTION THROUGH PURKINJE SYSTEMS.

The impulse, after passing through the right and left bundle branches, passes into the Purkinje fibres and also its multiple ramifications within the subendocardial surfaces of both ventricles. The impulse then travels from the endocardium to the epicardium of ventricular muscle perpendicularly.

CONDUCTION THROUGH VENTRICULAR MUSCLE.

In human beings, the midportion of the interventricular septum is activated normally in a left to right direction. So the depolarisation of the ventricular muscle begins at the left side of the interventricular septum because the Purkinje fibres arise more proximally from the left bundle branch than from the right bundle branch and activates the left side of the septum initially. After midseptal activation from the left to the right direction (FIG. 3), the impulse comes down the septum to the apex of the heart and next portions of myocardium that is activated is the anteroseptal region of the ventricular myocardium (FIG. 4). The impulse then proceeds along the right and left ventricular walls to the atrioventricular groove. The spread of excitation through the ventricle proceeds from the endocardium to the epicardium and thus the whole of the right and left ventricular walls are depolarised (FIG. 5). The portions of the ventricles that are excited lastly are the posterobasal regions of the left ventricle, the pulmonary conus, and the uppermost portion of the interventricular septum (FIG. 6).

CELL-TO-CELL CONDUCTION.

Earlier conception was that there is protoplasmic continuity between cells of the cardiac muscle and thus impulse

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is transmitted through the intercellular bridges. But electron microscopic studies reveal that there is no such bridges or protoplasmic continuity, the cells are bounded on all sides by membranes of high resistance. Transmission of impulse through this membrane is impossible. But the intercalated disc which crosses the short axes of the cells offers very low resistance and impulses reaching the intercalated disc are quickly propagated to the cells. So the syncitium-like properties of cardiac muscle are due to presence of low resistance intercalated discs (FIG. 7).

INTRACELLULAR CONDUCTION.

Through the specialised areas of the intercalated discs, the impulse ultimately reaches the cell membrane - sarcolemma. From here, the impulse is transmitted quickly through the transverse tubules (T tubules) - sarcolemmal invagination. This T tubule passes through the Z-line and here it is in close apposition with the sarcoplasmic reticulum that is present in the area in between the Z-lines. So the impulse from the cell wall is transmitted through the transverse tubules and then through the sarcoplasmic reticulum, and reaches ultimately to the contractile units of the muscle (FIG. 7).

BASIC PROPERTIES OF CARDIAC MUSCLE

The properties present in other muscles are also shown by the cardiac muscle. But it shows certain special features. They are briefly summarised below:

1. RHYTHMICITY.

One of the main characteristic features of the cardiac muscle is that it can initiate its own impulse rhythmically. This inherent rhythmical property is present throughout the cardiac muscle as evident from the electrophysiological studies of the single fibre from the S.A. node, A. V. node, atrial muscle, Purkinje fibre and also from the ventricular muscle fibre. If a strip of muscle fibre from the atrium or ventricle is perfused in normal physiological solution with proper ionic concentrations, pH, temperature, etc., then it beats

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rhythmically, proving the presence of pacemaker activity. It has been discussed earlier that the rate of rhythmicity in the S. A. node is 70 to 80 per minute, in A. V. node 40 to 60 per minute, in atrium 60 per minute, in ventricle 20 to 40 per minute. Due to higher rhythmical property of the S. A. node, it controls the rest of cardiac muscle and thus heart beats at the rhythm of the S. A. node. When the S. A. node fails, the A. V. node takes the charge and if it fails, the atrium and afterwards ventricle take the charge of maintaining heart beat.

Transmembrane potentials, recorded simultaneously from the single fibre of the S. A. node, A. V. node and ventricle during normal beating, vary in their successive phases from those are recorded separately in isolated preparation. Difference in membrane potential recorded in between outside and inside cell membrane is known as transmembrane potential. Transmembrane potential can be recorded by inserting micro-electrode (0.2 p) directly into the single cell and the indifferent electrode is kept outside the cell. As soon as the micro-electrode penetrates the cell membrane and if the indifferent electrode is kept outside the cell, a potential difference ranging from - 80 to - 90mV will be shown in the galvanometer. The resting transmembrane potential, thus recorded in the S. A. node is -80 mV, but the same, in the A. V. node, atrial muscle and ventricular muscle is -90 mV. This resting membrane potential will be maintained until the resting state is disturbed by propagated impulse. With the onset of excitation, the steady state is changed rapidly and the membrane potential attains to + 20 mV. After reaching at this level, it maintains steady state for a while and gets down gradually to its initial resting state. The upward deflection is the depolarisation phase as the membrane permeability to Na^+ is altered due to excitation. In resting state the extracellular Na^+ concentration is higher than that of the intracellular and the permeability of Na^+ to the cell membrane is hastened during excitation. So during depolarisation, there is an influx of Na^+ and this process is maintained until it reaches + 20 mV. Here Na^+ entry is decreased and K^+ begins to come out from the cell. This phase is known as repolarisatiom. Heart remains in a state of systole at this stage. K^+ efflux

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decreases after reaching the resting level and reorientation of ions (K^+ and Na^+) takes place at this stage by 'pumping', against electrochemical gradient and by metabolic energy. This phase is known as slow diastolic depolarisation. This phase coincides with the period of diastole. This process is very slow and due to reorientation of ions, the threshold potential (-60 mV) is slowly achieved and the membrane is further depolarised and the process is repeated. This process once started is maintained in the cardiac muscle until death ensues. How this process is initially being started in embryonic cardiac tissue is not yet fully known but it is presumed that certain hypoxic state may have been the cause of excitability in the embryonic stage. This slow diastolic depolarisation phase is the characteristic of pacemaker activity. This slow diastolic depolarisation phase of transmembrane potentials is absent in other than pacemaker area if the same is recorded simultaneously from single cell in a pacemaker-dominated heart. This phase is however present in a transmembrane potential recorded from any isolated preparation of cardiac muscle.

Cardiac rhythm is altered following stimulation of vagi or sympathetic nerve supplying the heart. The cause of the slowered heart rate following vagal stimulation is presumably due to prolongation of the slow diastolic depolarisation phase and also due to hyperpolarisation for increased permeability of K^+ to the cells. The rate of firing is decreased and longer time interval is required to achieve the level of threshold potential. Strong vagal stimulation causes the complete disappearance of spontaneous discharge for some time. These effects are in the vagal nerve endings due to liberation of acetylcholine, which causes hyperpolarisation of the cell membrane by increasing the K^+ permeability. On the other hand, cardiac sympathetic nerve stimulation or administration of adrenaline induces the membrane potential to fall more rapidly and the rate of spontaneous discharge increases greatly.

2. CONDUCTIVITY.

Conduction of impulse through different parts of the heart has already been discussed in detail. The impulse

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originated at the S. A. node spreads over the atria and reaches the A. V. node through the internodal fibres. There is no special connecting tissue between the two nodes, demonstrated histologically but from electrophysiological studies, the existence of such tissue has been reported by Carvalho and others (1961). The A. V. node transmits the impulse through the bundle of His and its branches to the ventricles. From the apex of the heart through the Purkinje fibres the impulse is conducted to the base. Conduction in the bundle of His and the Purkinje fibres is 1 metre per second, still less in the ventricular muscles 0.4 metre per second and least in the S. A. node 0.05 metre per second and A. V. node 0.1 metre per second.

3. EXCITABILITY AND CONTRACTILITY.

Like other muscles, the cardiac muscle is excitable by adequate stimuli and responds by contraction. The fundamental contractile unit of the cardiac muscle is myofibril which contains the protein units, action and myosin. During contraction these two units are associated in presence of ATP and thus the fibre is shortened, but during rest these are dissociated again with the resynthesis of ATP. Myosin itself is an enzyme 'ATP-ase' capable of dephosphorylation of ATP. Ca^{++} ion activates the ATP-ase activity - favouring prompt association of actomyosin and ADP complex. Excess calcium always keeps the muscle unit in contracting state (Calcium rigor) due to association of more contractile units. K^+ ions do not favour association of actin and myosin (Szent-Gyorgyi and Hajdu, 1952). So if excess K^+ is added in the extracellular fluid then the heart muscle gradually stops in diastole.

4. ALL-OR-NONE RESPONSE

If a quiescent heart muscle is stimulated at widely spaced electrical shocks of increasing strength then muscle contracts as a whole only when the threshold strength is reached. But there was no such increasing amplitude of contraction with increasing intensities of stimulation. This was observed by Bowditch (1871). Single skeletal muscle fibre behaves like this but if the entire muscle is stimulated with graded intensities of stimuli then graded responses are encountered.

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5. REFRACTORY PERIOD.

This is another characteristic property of the heart muscle. The refractory period of the heart is long and can be divided into three parts : (1) Absolute refractory period - This period extends throughout the whole period of contraction. Any stimulus, however strong, will fail to elicit a response if it falls within this period. For this reason, heart muscle cannot be tetanised. This long refractory period ensures enough time for recovery of the cardiac muscle. This is the reason why cardiac muscle cannot be fatigued. This period coincides the period from the onset of depolarisation phase to the repolarisation up to the threshold potential (FIG. 170). (2) Relative refractory period - This starts immediately after the absolute refractory period and involves the first part of relaxation. Only a very strong stimulus will be effective. This period begins when the transmembrane potential during repolarisation phase has just reached the threshold potential (-60 mV) and ends just before the repolarisation phase is ceased. (3) There is another type of refractory period observed after the relative refractory period which is known as supernormal period. This period is limited from the point of termination of repolarisation to the beginning of slow diastolic repolarisation phase.

6. TONE.

Heart muscle possesses tone. This tone is independent of nerves and can be adjusted. In this way, it can maintain a fairly constant tension upon its varying contents.

CARDIAC CYCLE

DEFINITION

Changes that occur in the heart during one beat, are repeated in the same order in the next beat. This cyclical repetition of the various changes in heart, from beat to beat, is called cardiac cycle.

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CARDIAC CYCLE TIME.

This is the time required for one complete cardiac cycle. With the normal heart rate of 75 per minute, this time will be 60/70 = 0.8 sec. It means that every event in the cycle will be repeated at the interval of 0.8 sec. It is obvious that the cardiac cycle time will be inversely proportional to the heart rate.

INTERRELATIONS OF THE VARIOUS EVENTS IN THE CARDIAC CYCLE.

In the cardiac cycle there are four main events: atrial systole, atrial diastole, ventricular systole, and ventricular diastole. All the other changes are subsidiary to them.

The atrial systole is the first event (0.1 sec.). It initiates the cardiac cycle, because the pacemaker S. A. node is situated here. Due to higher atrial pressure, the first half of atrial systole is stronger than that of the last half. After systole comes the atrial diastole (0.7 sec.). These two alternately follow each other and constitute the atrial cycle (0.8 sec.).

Just after the atrial systole, the ventricular systole (0.3 sec.) begins and is immediately followed by its diastole (0.5 sec.). These two events repeat alternately and make up the ventricular cycle (0.8 sec.).

At the onset of ventricular systole, the A. V. valves close producing the first sound. The semilunar valves open a little later. The interval between the closing of the A. V. valves and opening of the semilunar valves, is called the isometric contraction period (0.05 sec.). During this period ventricles contract as closed cavities and intraventricular pressure steeply rises.

After this phase, comes the ejection period - when blood is pumped out of the ventricles. The first part of this period, when the outflow is very rapid, is called the maximum ejection period (0.11 sec.). The second part, when the rate of flow slows down, is known as the reduced ejection period (0.14 sec.). Here, ventricular systole ends and diastole begins.

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At the beginning of ventricular diastole, the semilunar valves close produring the second sound. There is a brief interval between the beginning of diastole and the closure of the semilunar valves - known as the protodiastolic period (0.04 sec.). So that, second sound occurs actually after this period. The A. V. valves open a little after the closing of the semilunar valves. The interval between these two is called the isometric relaxation period (0.08 sec.). During this period ventricles relax as closed cavities and intraventricular pressure steeply falls. At the end of this period, the intraventricular pressure goes below that of the atria and the A. V. valves open. Atrial blood rushes into the ventricles. producing the third sound. Here, ventricular filling begins. The first part of filling is very rapid, being known as the first rapid filling phase (0.113 sec.). The maximum filling takes place during this brief period. The intermediate part of filling is very slow and is known as diastasis or slow inflow phase. Although this is the longest phase (0.167 sec.), yet the amount of filling is minimum. The last part of diastole corresponds with atrial systole. Due to active contraction of the atria, filling becomes very rapid. This last rapid filling phase (0.1 sec.) is responsible for the last part of ventricular filling. Due to rapid rush of blood, another sound is produced - the so - called fourth sound of heart. Here, ventricular diastole ends and systole commences again. In this way the cycle continues (FIG. 8).

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NERVE CONDUCTION

by Dr. Jayanta Kumar Panda

The importance of nervous system lies in the fact that it controls and integrates the different bodily functions, and acts as the chief coordinating agency in the animal body. The nerve tissue is differentiated into two lines (i) neural cells - neuroblasts, and (ii) glial cells - spongioblasts. The glial cells or neuroglia perform as interstitial tissue in grey and white matter whose main function is support, insulation and phagocytosis, whereas the neuroblasts pass through successive stages and ultimately give rise to the matured neurone.

Neurone :

A nerve cell with all its processes is called a neurone (Fig. 1). It is the structural and functional unit of nervour system. It may consist of a nerve cell body or soma and two types of processes - axon and dendrite. Dendrites branch extensively close to the cell body and carry excitation to the cell body, whereas axons spread excitation away from the cell body. The structures contained in the cell body viz. (i) nucleus, (ii) neuroplasm with neurofibrils passing through the neuroplasm from dendrite to the axon, and nissl bodies (granules), (iii) mitochondria, (iv) golgi apparatus, (v) ribosome, (vi) endoplasmic reticulum, (vii) centosome, (viii) cell inclusions and (ix) neurosecretory materials.

PHYSIOLOGY OF NERVE CONDUCTION

Resting potential :

In a state of rest there is a difference in potential of the order of 60-90 millivolts between the outer surface of a cell and its protoplasm, the cell surface being electrically positine with respect to the protoplasm.

Origin of resting potential :

According to Hodgkin and Huxley (1952) bioelectrical potentials are caused by inequal concentration of pottassium,

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sodium and chlorine ions within the cell and outside it and by the variable permiability of the surface of the membrome to them.

The protoplasm of nerve and muscle cells contains 30-50 times as many pottasium ions, 8-10 times fewer sodium i and 50 times fewer chlorine ions as does the extra To the structural elements of the membrome are bound various i which lend a particular electric charge to the walls of its p there by impeding or facilitating the passage of other ions. I is supposed for example, that the presence of dissociated phosphate and carboxyl groups is the reason only the membrome nerve fibre is much less permiable to anions than to cations. Permiability to different cations also various and charges re in the different functional conditions of the tissue. At rest the permiability of nerve fibre membrome to pottasium ion is between 20 to 100 times that to sodium ions, where as in an ex state the ratio is significantly reversed.

***ACTION POTENTIAL :**

If a sufficiently strong stimulus (for instance electri shock) is applied to part of a nerve fibre, it will give rise t excitation, the main manifestation of which is a rapid variati of the membrome potential, which is known as action potential.

* (During the resting state, the potassium ions, due to their higher concentration, tend to diffuse out ward and this creates an internal negativity. However, the internal negativi becomes great enough to prevent further movement of pottasium i and thus an equillibrium is reached. This is the resting poten A cross the resting plasma membrome the resting potential is usually about 60 to 70 mV .)

1. **EXCITABILITY.** The nerve fibre can be stimulated by a suitable stimulus, which may be mechanical, thermal, chemical or electrical. In experiments, electrical stimulation is usuall employed because its strength and frequency can be accurately controlled. The following changes will show that a nerve has be excited: (a) The muscle or the gland where the nerve ends will

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respond. (b) The stimulated spot on the nerve becomes electri-ally negative and this wave of negative potential passes along the nerve and can be detected by galvanometer or by CRO. (c) The action potentials - An electrical disturbance always accompanies the travelling nerve impulse. In resting cell the surface is positively charged and the interior is negatively charged. When the surface is stimulated and the permeability is increased as a result there is reversal of polarisation. The surface at the stimulated point becomes negative (cathode) causing catelectrotonic change. When this change rises to threshold level, impulse will pass like selfpropagated disturbance by drawing positively charged particles from the neighbouring points which in turn becomes cathode. The depolarisation of the membrane is the first step of the manifestation of an impulse. After an initial slow rise, depolarisation wave overshoots rapidly and reaches the isopotential line (zero line) to approximately + 35 mV. After that it reverses and begins to fall very rapidly towards the resting level (- 70 mV). At approximately two-thirds of repolarisation, the rate of fall is being abruptly slowed. This slower fall is known as negative after-potential (after-depolarisation). The rapid rise of depolarisation wave and the rapid fall of repolarisation wave are known as spike potential. After reaching the basal level the wave overshoots slightly but slowly in the hyperpolarising direction. This is known as positive after-potential (after-hyperpolarisation). The whole sequence of potential changes in the nerve following excitation is known as action potential or membrane potential (FIG. 2).

MECHANISM OF THE DEVELOPMENT OF ACTION POTENTIAL

In resting state the nerve fibre remains in polarised state and the membrane potential lies within - 70 mV. The inside of the nerve is negative and the outside of the nerve is positive. Na^+ concentration outside the membrane is higher than that of inside the membrane. K^+ concentration inside the membrane is also higher than that of outside the membrane. K^+ can permeate through the membrane at resting state but the Na^+ cannot permeate. rmeability of Na^+ to membrane is increased only after excitation

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and it is the first event of the action potential. The action potential occurs in successive stages of depolarisation, repolarisation, negative after-potential and positive after-potential. It has been postulated that in resting state calcium ions (Ca^{++}) remain binding to the protein surfaces of the membrane pores and it does not allow Na^{+} to permeate through these resting pores. During excitation Ca^{++} is dislodged from its binding site and the permeability to Na^{+} is increased. So the depolarisation starts with the onset of Na^{+} entry and thus an increase in Na^{+} conductance is taken place. The tremendous increase is Na conductance during this period is known as activation of membrane. Due to this, the reversal of potential is caused with the development of positivity inside the membrane and negativity outside (FIG. 3). But with the increase of positivity inside, further entry of Na^{+} is prevented and calcium begins to bind with the proteins of the membrane pores. But as soon as the action potential attains the voltage approximately + 35 mV, K^{+} begins to come out from inside the membrane. The inside becomes negative and outside becomes positive again (FIG. 4). This stage is the repolarisation phase and K^{+} conductance is increased to the maximum. The mechanism underlying the process of K^{+} conductance is mostly hypothetical and increased positivity inside the membrane due to Na^{+} entry during depolarisation phase, allows the K^{+} to come out and the resting potential is slowly achieved. But at the later period of this phase (at the termination of spike potential) K^{+} conductance is slowed down and thus a few milliseconds are delayed in restoring the membrane potential. This state is known as negative after-potential which has been described to be the cause of increased K^{+} concentration outside the membrane. This increased K^{+} concentration may hinder further efflux of K^{+}. With the disappearance of the negative after-potential, though the resting membrane potential is achieved yet the resting ionic status is not established. It is achieved by the active Na^{+} pump mechanism and Na^{+} begins to come out from inside the membrane creating negativity again (Figs. 5). The positive after-potential is due to this process of Na diffusion from inside to outside the membrane. The negativity, produced due to active Na^{+} pump mechanism, causes the K^{+} to diffuse back to the interior of the nerve fibre. For the active Na^{+} and K^{+} pump mechanism high energy phosphate (ATP) is required. In this way resting normal ionic status is established during the period of positive after-potential.

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Excitablity depnds upon the following factors : (i) strength of stimulus - A minimum strength is essential. (ii) Duration of stimulus - The stimulus must continue for a certain minimum period, which varies inversely as the strength. Excitability of a nerve fibre can be determined by studying its strength-duration relationship (threshold stimulus intensity duration) of the stimulus. Current intensity of stimulus which is just adequate to cause an impuse is called the threshold. Intensity below the threshold is known as subliminal. Magnitude of current just sufficient to excite a nerve or muscle is called rheobase and the minimum time required to have a response is known as utilisation time. The shortest duration of current flow which will excite the nerve or muscule under current strength equal to twice the rheobase is called the chronaxie. Chronaxie value is a useful index of the relative excitability of the tissues. (Fig. 6)

2. CONDUCTIVITY : The nerve impulse is conducted along the nerve fibre. Conductivity shows the following characteristics: (i) Impulse is propagated along a nerve in both directions. (But under normal conditions the nerve impulse travels in one direction only - in the motor nerve towards the responding organ ; in the sensory nerve towards the centre. This is due to the action of 'synapse'.) (ii) Velocity of nerve impulse - The nerve impulse is propagated with a definite speed (other conditions remaining same). The conduction velocity depends upon the diameter of the nerve fibres, the thicker fibres showing higher velocity. The conduction velocity also depends upon the presence or absence of myelination and also on temperature.

FACTORS AFFECTING CONDUCTIVITY AND EXCITABILITY:

(a) Temperature - cooling diminishes and warming increases these properties. (b) Mechanical pressure-depresses conductivity and excitability. (c) Blood supply - CO_2 and narcotics, viz., ether, chloroform, alcohol, novocain, etc., diminish and finally abolish excitability and conductivity. (e) H-ion concentration - increased pH (alkali) increases and decreased pH (acid) diminishes. At pH 8.0 the nerve becomes

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hyperexcitable and spontaneous discharge may occur. Even a single stimulus may cause multiple response (repetitive response (f) Effect of ions - lack and excess of Ca have the same effects as rise and fall of pH respectively. Changes of K^+ exert opposite effects. Na^+ and Mg^{++} - similar to K^+ but less in degree (hence, Na^+, K^+ and Mg^{++} - are neuro-excitatory, while Ca^{++} is neurosedative). (g) O_2 lack-depresses and if continued abolishes these properties. If O is readmitted, they return.

3. ALL-ON-NONE LAW : If the stimulus be adequate a single nerve will always give a maximum response. If the strength or duration of the stimulus be further increased no alteration in the response will take place. This property is present in single fibre preparation. In the whole nerve this property is different.

4. REFRACTORY PERIOD : When the nerve fibre is once excited, it will not respond to a second stimulus for a brief period. This period is called absolute refractory period. The absolute refractory period means that the nerve is completely refractory to stimulation-in other words is incapable of eliciting an action potential at any intensity of stimulation. During the absolute refractory period there is total inactivation of the sodium [illegible] carrier mechanism and as the Na ions cannot enter the fibre, there is no development of the action potential. Immediately following this, there is a brief relative refractory period, during which the excitability is subnormal but gradually rising. This is succeeded by a third brief period of increased excitability, known as supernormal phase. Lastly, there is a period of subnormal excitability-subnormal phase.

5. SUMMATION : (Latent addition). In a nerve fibre summation of two submaximal stimuli is possible.

6. ADAPTATION : The nerve fibre quickly adapts itself. Due to this adaptation there is no excitation during the passage of a constant current. Only when the strength of the current is suddenly altered or the current is made or broken excitation takes place. A gradual change will fail to excite.

Contd...P/7.

7. ACCOMMODATION : If a stimulus even with stronger strength is applied very slowly to a nerve, then there may have no response only due to lack of attaining the threshold strength. This phenomenon is called accommodation, i.e., slowly applied stimulus is accommodated by the nerve no matter how strong the stimulus is applied.

8. INDEFATIGABILITY : In the nerve muscle preparation, if the nerve is stimulated repeatedly, then after a certain period the muscle fails to give any response. Now if that nerve is isolated from the muscle and placed on a fresh muscle, then application of stimulus will excite the muscle. This shows that nerve is not fatigued.

THE FOLLOWING IS A BRIEF SUMMARY :. (i) Unlike muscles, the excitability, conductivity 2nd the recovery process can go on in a nerve for a considerable period even in absence of oxygen. (ii) The chemical changes in the nerve are roughly of the same nature as seen in the muscles. Pyruvic acid is formed and if O_2 supply be insufficient, lactic acid accumulates (same as in muscles). Thiamine which is essential for complete oxidation of these acids, is found in good amount in the nerve fibres. Although carbohydrates burn, yet they are not the only source of energy (contrast with nerve cells-which possibly use galactose). The breakdown of phospholipids also takes an essential part here. It is said that, the energy requirement of the resting nerve is supplied by combustion of sugar and phospholipids mainly. During activity, ATP and creatine phosphate break down and supply energy for the propagation of the nerve impulse. Both ATP and creatine phosphate are then resynthesised but the source of energy of this recovery process is not known (may be in the same way as in muscles). (iii) During activity acetylcholine is liberated by the cholinergic fibres, while norepinephrine by the adrenergic fibres. (iv) The nerve fibres are rich in K^+ and thiamine. During activity K^+ (and possibly thiamine) diffuses out and Na^+ and enters the fibres. This migration of K^+ and Na^+ seems to be intimately related to the properties, viz., excitability, conductivity, etc., of the nerve fibres.

Contd...P/8.

EXCITATION AND THE LOCAL CIRCUIT THEORY

It was suggested long ago that propagation of a nerve impulse depends on the flow of current in local circuits ahead of the active region, which depolarizes the resting membrane, and causes it in turn to become active. In this local, circuit theory, the flow of current from region A to region B (Fig. 7) results in movement of the active region towards the right. In both myelinated and non-myelinated fibres the principle is the same in that the active region triggers the resting region ahead of it by causing an outward flow of electric current.

CORE CONDUCTOR THEORY OF NERVE IMPULSES

If electrodes are applied to the surface of a nerve fibre, electronic potentials develop on the fibre at the electrodes and in their vicinity. The potentials are the greate underneath the electrode and fall off at points farther away. The development of these potentials and their associates is explained by considering the nerve fibre to act as a core conductor; a cylinder of conducting fluid material (axoplasm) with a sheath (cell membrane) of high electrical resistance, surrounded by a layer of conducting medium.

SALTATORY CONDUCTION OF NERVE IMPULSES

Conduction of nerve impulses is very rapid in myelinated nerve fibres in comparison to those without a myelin sheath. The myelin sheaths are interrupted regularly at spaces known as the nodes of Ranvier. At these nodes the membrane of a neuron makes contact with the surrounding fluid. The distance between successive nodes is more in neurons of large diameter than in neurons of small diameter. Conduction in myelinated neurons is supposed to take place by jumping or skipping of the action potentials from one node to another instead of travelling along the nerve membrane. Such skipping movement from node to node is called saltatory conduction (Fig. 7)

SYNAPTIC TRANSMISSION

The synapse, according to Sherrington (1898), is the functional connection between two neurons. Between the pre-

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synaptic and post-synaptic membranes a cleft of several angstrom units is present.

ELECTRICAL THEORY OF SYNAPTIC TRANSMISSION

In the crayfish giant motor fibre, it has been demonstrated that impulses pass from the pre-synaptic portion to the post-synaptic membrane. The depolarization here spreads across the synapse from the pre-synaptic portion and is measureable in the post-synaptic membrane. Any depolarization in the post-synaptic fibre does not cause hyper-polarization of the pre-synaptic portion indicating an undirectional flow of current. Thus, the synapse acts as a rectifier with a high resistance towards flow of current in the opposite direction. This type of conduction may also take place in other animals.

CLASSIFICATION OF SYNAPES

On the basis of the nature of connections between neurons, synapses have been classified into three types.

AXOSOMATIC SYNAPSES. In this type, the terminal processes of the presynaptic neuron end on the cell body or soma of the post-synaptic neuron. This type of synapses are found in the cerebellum between the basket cells and Purkinje cells.

AXO-DENDRITIC SYNAPSES. The terminal processes of the axon of the presynaptic neuron end in the dendrites of the post-synaptic neuron on (Fig.8). In the cerebellum, the climbing fibres from connections with dendrites of the Purkinje cells.

AXO-AXONIC SYNAPSES. If the terminal processes of the presynaptic axon make connections with the terminal processes of the post synaptic neuron, this type of synapses are known as the axo-axonic synapses.

STRUCTURE. Electronmicroscopic studies have revealed that the axons of the presynaptic neurons end in expanded terminals-the synaptic or terminal knobs or buttons. The membrane of the synaptic knob is the presynaptic membrane while that of the cell body is the postsynaptic or subsynaptic membrane. Though there is intimate contact between the two membranes, they are separated by a gap, the synaptic cleft

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measuring about 10-20 nm. Thus, there is no physical continuity in the cytoplasm of the presynaptic, and postsynaptic neurons at the synapses. However, at some synapses, the two membranes show areas of closer proximity and the outer layers of the unit membranes of the pre and post synaptic membranes even fuse, forming gap junctions found in the spinal neurons of teleost fishes and giant fibers of cray fish. In brain cortex parallel intersynaptic filaments (Fig. 9) extending between the pre and subsynaptic membrane in the synaptic cleft have been demonstrated by D. Roberits (1961). The function of the filaments is not yet known. In same synapses (brain cortex and retina), the filaments form a web like network on the subsynaptic membrane extending even into the Cytoplasm of the postsynaptic neuron. This network is known as the subsynaptic web. The Cytoplasm of the terminal knobs contains mitochondria and synaptic vesicles. The synaptic vesicles are spherical or ovoid shaped structures with diameters ranging between 20-65nm. The vesicles are bound by a 4-5 nm thick unit membrane. The vesicles are more concentrated towards the synaptic cleft. The synaptic vesicles contain the excitatory neuro-transmitter substances that mediate transmission of nerve impulses from the presynaptic to postsynaptic neurons. The vesicles may be found on both sides of the synaptic junction, and in electrically transmitting neurons also.

CHEMICAL THEORY OF SYNAPTIC TRANSMISSION

The transfer of a nerve impulse across a synaptic junction is known as synaptic transmission. This process is believed to prought about by the release of chemical substances at the synapse and takes place in the following steps -

1. RELEASE OF THE NEUROTRANSMITTER SUBSTANCE. The neurotransmitter substances synthesized in the terminal processes of the axons are stored in the synaptic vesicles. On arrival of an nerve action potential through the axon into the terminal knobs, by some unknown mechanism, the vesicles release the neurotransmitter substance. Calcium ions are required for the release, and magnesium inhibts the process. After the release of the transmitter the vesicular membrane moves into the cell cytoplasm and is used to package new transmitter substance synthesized.

Contd...P/11

2. DIFFUSION OF THE TRANSMITTER SUBSTANCE. The neuro transmitter substances released by the presynaptic terminal diffuse across the synaptic cleft and bond to specific receptor sites on the subsynaptic membrane. In the vertebrate neuromuscular junction acetyl choline is released from 100-300 presynaptic sites and diffuses across a distance of less than 1 p m.

3. PERMEABILITY ALTERATIONS IN THE SUBSYNAPTIC MEMBRANE. The binding of the neurotransmitter to the receptor molecules is accompanied by alterations in the permeability of the subsynaptic membrane. Two types of alterations in the permeability are observed. The first is a general type in which the permeability of the postsynaptic membrane to all types of ions bringing about a depolarization of the membrane and excitatory post synaptic potential is produced. The second type increases the permeability of the membrane to K^+ and chloride ions causing hyperpolarization of the membrane and inhibitory post synaptic potential. If the synaptic potentials are great enough to produce sufficiently strong local currents, a spike is generated in the appropriate region of post synaptic neuron.

4. DESTRUCTION OF THE NEUROTRANSMITTER SUBSTANCE. The neurotransmitter substance is destroyed quickly so that normal subsynaptic resting potentials are restored and the neuron may respond again to a new stimulus.

CHEMICAL NATURE OF TRANSMITTER SUBSTANCES. Acetylcholine has been considered to be the transmitter substances of the (1) cholinergic effector organs, (2) postganglionic autonomic nerve endings (3) preganglionic sympathetic nerve endings, (4) at some sites of the CNS, (5) neuromuscular junction.

NATURE OF TRANSMISSION IN THE CNS

CHOLINERGIC TRANSMISSION. Role of acetylcholine (ACh) in the transmission of nerve impulse in the cholinoceptive synapses at the neuromuscular junction, autonomic ganglia, etc., is well known (Fig. 10). The presence of ACh in the CNS has been known for last four decades. Being distributed non-homogeneously it

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performs a specific function in some region but not in all regions. Little ACh is present in the dorsal root, the optic nerves, and the crebellum. Brain stem, thalamic nuclei and cerebral cortex contain moderate amount, but caudate nucleus and the retina contain very large amount. Nature of distributio of true acetylcholinesterase (AChE) is roughly parallel to that of ACh. Studies with the multiplebarrelled micropipette it is observed that transmission in the recurrent axon synapses upon Renshaw cells is cholinergic in nature and it resembles those in neuromuscular junction and in postganglionic cells in the autonomic ganglia. With studies in central neurones, it is observed that 10% of cortical neurones are activated by cholinergic synapses. Pseudocholinesterase is also present in various parts of the CNS.

CENTRAL ADRENERGIC TRANSMISSION. Norepinephrine is the major transmitter agent in the postganglionic sympathetic neurones. Its presence in the ganglia can be demonstrated by histofluorescent methods.

The presence of adrenergic substances in the extracts of the mammalian brain and also its regional distribution in the brain have been described as early as 1954 and indicated that adrenergic transmission is possible. Whole brain concentrations of catecholamines are about 0.1 to 0.5 pgm per gm. Midbrain, pons, medulla, cerebral cortex, hippocampus, cerebellum and spinal cord contain catecholamines in lower concentration. In the hypothalamus, olfactory bulb, retina, median eminenca, limbic system, large amounts of amines are present. In the spinal cord, there is a system of descending adrenergic fibres which terminate about neurones of the intermediolateral horn. Central adrenergic systems have also been mapped out by following the technique of fluorescent.

Mechanisms of synthesis, storage, inactivation and removal of norepinephrine are present in the CNS and are in general similar to those in peripheral adrenergically innervated tissues. As catecholamines cannot cross the blood-brain barrier, the enzymes and precursors required for norepinephrine synthesis are present in the brain.

DOPAMINE, the immediate precursor of norepinephrine is also suspected to act as transmitter agent in certain motor function of the CNS.

PLANT GROWTH HORMONES

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A plant hormone is an organic compound synthesized in one part of a plant and translocated to another part where, in very low concentration, it causes a physiological response. The response in the target organ is not necessarily promotive; there are plant hormones that are seen to have inhibitory effects on physiological processes like growth and differentiation. Basing on their action two types of plant growth hormones have been recognised: growth promoters and growth inhibitors. Inorganic ions e.g. K^+ or Ca^{2+} which have well known growth responses are not growth hermones. Neither are synthetic organic compounds like 2,4 - D considered as hormones - such synthetic organic compounds with hormone like effect are known as plant growth regulators. A hormone must essentially be synthesized and translocated within the plant. Hormones are also required in very minute doses usually concentrations of μM or less. There are still only five groups of well accepted hormones. They include Aux~~e~~in, gibberellins, cytokinins, obscisic acid and ethylene.

THE AUXINS

The term auxin (GK Auxein = to grow) was first used by Frits Went when he observed the curvature of oat (*Avena sativa*) coleoptiles towards light. This curvature phenomenon, called phototrapism, was explained to be the result of a substance present in the coleoptile tip. The substance which was named as 'auxin' could diffuse from cut tips to agar blocks when placed below. The agar block could new replace the effect of the coleoptils tip (Fig.1).

Went's auxin was later identified as indole -3-acetic acid (IAA, Fig.2) Subsequently the word 'auxin' was used as a generic name to describe all compound having effects similar to IAA. 4-chloro-indoleacetic acid (4-chloro IAA) and Phenyl acetic acid (PAA) are auxins widespread among plants. Similar compounds like naphthaneacetic acid (NAA), undole berlhrie acid (IBA), 2,4 - dichloro phenixy cocetic acid (Fig.2) synthesised and having physiological responses common to IAA are also considered to be auxins, but as mentioned above these ar not growth hormones but classified as plant growth regulators.

IAA, one of the common naturally occuring auxin is synthesised in young tissues such as shoot meristems and growing leaves. It is synthesised from the amino acid trypto-phan and is transported in a basipetal direction normally via parenchyma.

Physiological Effects of Auxins:

According to the vast array of literature available regarding auxin , the effect of auxins is stimulatory in some cases and inhibitory in other. Some of the important function are discussed below.

a) Cell elongation: Auxins ~~induce~~ induces cell elongatio by in tiving cells especially in the young cells.

b) Phototropism: The differential growth of shoot tips which causes phototropic movement of the shoot is because of unequal distribution of auxins in illuminated and non illuminated side which is probably accomplished by light-reduced inactivation of auxin or transport of auxins from illuminated to the non-illuminated side.

c) Geotropism: If an intact seedling is placed in a horizontal position, it will respond to the earth's gravitational field* with a particular pattern of growth. The positively geotropic movement of the root and negatively geotropic movement of the shoot is explained by the transport of auxins away from the gravity in the roots and towards gravity in stems.

d) Root initiation: Application of auxins to cuts ends of stems initiates the differentiation of adventious roots in cuttings - Auxins are also used in tissue -culture media for differentiation of the callus.

a) Parthenocarpy: Exogenous manipulation of auxins induces parthenocarpic development of fruits.

b) Abscission: The presence of auxins prevents the formation of abscission layers in fruits and leaves.

GIBBERELLINS

The gibberellins are another class of compounds whose minute quantities profoundly stimulate the growth of many plants.

DISCOVERY

The gibberellins were discovered in an interesting and incidental way. In early part of the twentieth century, Japanese farmers noted that some plants in rice fields were taller, thinner and paler than the normal plants and were sometimes devoid of fruits too. They named this disease as "bakanae", meaning foolish seedlings. Sawada (1912) suggested that the disease is due to a 'substance' secreted by a parasitic fungus, Fusarium moniliforme (syn. Gibberella

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fujikuroi) infecting the diseased plants. This suggestion was experimentally supported by Kurosawa (1926) who demonstrated that sterile filtrates of the fungus could initiate symptoms of bakanae disease in healthy rice seedlings. Later in 1939, Yabuta and Hayashi isolated this growth promoting substance in crystalline form and named it as gibberellin A, which has now been shown as a mixture of many growth promoters collectively known as gibberellins.

Since that time, gibberellins and allied substances have been found in higher plants also by Mitchell et al. (1951), West and Phinney (1957) and Sumiki and Kawarada (1961).

DEFINITION

A gibberellin (abbreviated as GA) may be defined as a compound which is active in gibberellin bioassays and possesses a gibbane ring skeleton (refer page 195). There are, however, other compounds (like kaurene) which are active in some of the assays but do not possess a gibbane ring. Such compounds have been called gibberellin-like rather than gibberellins.

ISOLATION, DISTRIBUTION AND BIOASSAY

About 29 gibberellins have been isolated so far and their chemical structures known. These have been named as gibberellin A_1 (GA_1), gibberellin A_2 (GA_2) and so on up to gibberellin A_{29} (GA_{29}). Of these Cross et al (1961) have isolated 6 gibberellins from the fungus. Fusarium moniliforme and designated them as GA_1, GA_2, GA_3, GA_4, GA_7 and GA_9. The same year, MacMillan et al isolated 3 gibberellins from bean seeds and named them as GA_5, GA_6 and GA_8. The GA_{10} and GA_{13} have been discovered by Mulholland (1963). All these compounds are sometimes referred to as constituting the gibberellin A series.

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Although the gibberellins wer originally isolated from a fungus, but now they have been shown to be present in almost all the groups of plant kingdom. For example, GA_1 and GA_5 have been isolated from immature seeds of Phaseolus vulgaris by West and Phinney (1959). Although all the organs of the flowering plants contain gibberellins, but the highest level has been detected in seeds. Young leaves and roots are also rich in them. It may, thus, be generalized that rapidly growing and developing regions of the plant possess higher concentrations of gibberellins.

The gibberellins can exist in more than one form within the plant. Hashimoto and Rappaport (1966) suggested that the esterified forms of gibberellins (i.e., neutral gibberellins) act as reservoir of active gibberellins. The active acidic form may be drawn from the neutral form as and when needed. In addition, bound forms of gibberellins also exist (mr Comb, 1961).

Many angiospermous plants have now been used as bioassay for gibberellins and gibberellin-like substances. A few of them are Avena sativa (lead section), Pisum sativum (intact seedling), Triticum vulgare (excised coleoptile) and Rudbeckia bicola (rosetted plants).

CHEMISTRY

The chemical structure of the gibberellins was established by Cross et al in 1961. They showed that the gibberellins (Fig. [illegible]) are a group of closely related compounds and possess a common feature, the gibbane ring skeleton. The gibbane ring consists of a carbon skeleton with 4 rings, designated as A, B, C and D. They are hence described

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as tetracarbocyclic compounds. Of the 29 gibberellins, 19 are C_{19} compounds and the other 10 have 20 carbon atoms each. Eighteen gibberellins are monocarboxylic, 7 are dicarboxylic and 4 are tricarboxylic acids. The various gibberellins differe from each other in the number and position of the functional groups present in the molecule. In gibberellin A_1, the functional groups are a carboxyl, one ethylenic double bond, two alcoholic hydroxyl groups (one secondary and the other tertiary), a saturated lactone and one methyl group. Gibberellin A_3 differs from GA_1 in the presence of one more ethylenic double bond in the ring A. It is, thus, more unsaturated than GA .

The gibberellin A_2 and gibberellin A_4 both have structures similar to that of GA_1 except for the difference in position of the tertiary hydroxyl group and the absence of a double bond in GA_2 and in the absence of tertiary hydroxyl group in GA_4. The gibberellin A_5 is, in fact, a dehydrogibberellin A_1 where the secondary hydroxyl group is eliminated from ring A, making the compound more unsaturated.

Gibberellin A_3 has been usually shown to be biologically most active followed by GA_1, GA_4 and GA_2 in descending order of their activity.

$$GA_3 > GA_1 > GA_4 > GA_2$$

PHYSIOLOGICAL ROLES

Gibberellins may be regarded as natural phytohormones on account of their wide range of distribution in plants and specificity of response of individual flowering plants to the exogenously applied gibberellins. The gibberellins, however, play important roles in the following processes :

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1. GENETIC DWARFISM. In certain plants, dwarfism is caused by the mutation of a single gene. Such individuals are called 'single gene dwarfs'. In these plants, dwarfism is due to shortening of internodes rather than a decrease in the number of internodes. Application of gibberellins on such dwarfs causes them to elongate so much as to become indistinguishable from the tall normal plants. Elongation of the stem, in fact, takes place due to an elongation in the internodes rathern than an increase in the number of internodes. Thus, genetic dwarfism has been successfully overcome by gibberellin A_3 treatment in many single gene dwarf mutants like *Pisum sativum*, *Vicia faba* and *Phaseolus multiflorus* (Brian and Hemming, 1955).

Two views have been put forward regarding the mechanism of control of dwarfism by gibberellins.

(a) It is due to the lack of endogenous gibberellins in dwarf plants or if at all present, they are in traces as to have no effect.

(b) A natural inhibitor is present in those plants which retard growth. And the gibberellin, when applied, nullifies the effect of this inhibitor.

2. BOLTING AND FLOWERING. 'Rosette plants' are characterized by their profuse leaf development and retarded internodal growth. But prior to the reproductive phase, their occurs striking elongation in the internode so that the plant attains 5 to 6 times the original height. Treatment of these 'rosette' plants with gibberellins, under conditions that would normally maintain the rosette form, induces them to bolting (or shoot elongation) and flowering (Lang, 1957). By regulating the amount of gibberellin applied, it is also

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possible to separate shoot elongation from flowering; with low dosages of gibberellins, the plant will bolt but not flower (Phinney and West, 1961).

It is, therefore, not amazing to find a direct correlation between the amount of gibberellin present and the habit of the plant, whether rosetted or bolted. Native gibberellin-like substances are found in higher concentrations in the bolted forms than in the nonbolted ones. This has been experimentally demonstrated in quite a few plants including the biennial Hyoscyamus niger by Lang (1957) and the cold-requiring plant Chrysanthemum morifolium and a long-day plant Rudbeckia speciosa by Harada and Nitsh (1959).

As far as the use of gibberellins in agriculture is concerned, it may be possible to grow cold-requiring plants in warm countries and long-day plants in short-day conditions at lower altitudes.

3. LIGHT-INDUCED INHIBITION OF STEM GROWTH. Light-grown plants reveal suppressed stem growth than the dark-grown (or etiolated) plants, indicating that light has an inhibitory effect on stem elongation. But this inhibitory effect of light on dyrm rlongation can be reversed at least in some plants (like Pisum sativum) by the application of gibberellins on these plants. This clearly suggests that endogenous gibberellin is the limiting factor in stem elongation.

Lockhart (1961) has given a possible explanation for it. According to him, exposure to light lowers the level of available gibberellins present in the plant. The lowered available gibberellin contents then, in turn, decrease the plasticity of cell walls, thus inhibiting stem growth. The theory has, however, not won the universal support on account of the following drawbacks :

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(a) Stem elongation is also induced in mustard seedlings, grown in dark, upon application of gibberellin.

(b) In some plants, gibberellin-stimulated stem growth has been found to be partially due to enhanced cell division and has nothing to do with cell wal plasticity.

(c) Germination of the seeds of _Lactuca sativa_ is not only promoted by gibberellins but by red light too.

4. _PARTHENOCARPY._ Like auxins, the gibberellins are also capable of inducing parthenocarpic fruit-set. Gibberellins are, in fact, more efficient than the auxins in inducing parthenocarpy. For example, Wittwer and Bukovac (1957) have found gibberellin to be about 500 times more effective than IAA in inducing parthenocarpy in tomatoes. Moreover, there are cases where auxins have failed to induce parthenocarpy while gibberellins are effective, as shown experimentally for apples (Davison, 1960) and stone fruits (Crane et al. 1960).

Gibberellin-induced parthenocarpy has been reported in many plants such as _Cucumis sativus_ (cucumber), _Solanum melongena_ (brinjal) and Zephyranthes sp. Whether the production of parthenocarpic fruits is a direct action of gibberellins or an interaction with the natural auxins of the plant has not been conclusively proved.

5. _BREAKING DORMANCY OF SEEDS._ The light-sensitive seeds (lettuce, tobacco) show poor germination in dark and on exposure to light their germination starts vigorously. But when these seeds are treated with GA_3, the light requirement is alleviated and they germinate in dark.

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6. BREAKING DORMANCY OF BUDS. In temperate areas, the buds produced in winter remain dormant until the next spring due to very low temperature. The dormancy in such cases is overcome by gibberellin treatment. Thus, GA_3 treatment to birch buds has replaced the light requirement for breaking dormancy (Eagles and Wareing, 1964). Gibberellins are also capable of breaking dormancy in potato tubers.

7. ROLE IN ABSCISSION. GA treatments have shown accelerated rate of abscission in explants of bean (Chatterjee and Leopold, 1964) and of Coleus (Gupta and Kaushik, 1969).

8. STIMULATION OF ENZYME ACTIVITY IN CEREAL ENDOSPERM.

Yomo (1960) and Paleg (1960) working independently showed that the gibberellins applied exogenously could stimulate amylase activity in isolated barley endosperm. It was then shown that it is the aleurone layer of the endosperm which sensitive to the gibberellin. Subsequent researches by Paleg (1964) and Varner (1964) revealed that GA treatment of isolated aleurone can cause release of the enzymes, amylase and proteinase. Finally, Jacobson and Varner (1967) showed that the two enzymes (amylase and proteinase) induced by GA treatment arise through de novo* synthesis. These enzymes participate in the breakdown of the stored starch to simple sugars. These sugars are then translocated to the growing embryo where they provide energy for growth.

9. SEX EXPRESSION. Gibberellins are also capable of altering the sex of the flowers. Galun (1959) could induce maleness by foliar application of GA_3 to the female flowers of Cucumis. Also, the antheridia have been induced to develop in many fern gametophytes by GA_3 treatment.

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* A Latin phrase, meaning anew.

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RELATIONSHIP BETWEEN AUXINS AND GIBBERELLINS

Auxins and gibberellins are similar to each other in that both promote cell elongation, flowering and parthenocarpy. These, however, differ from each other in many of the physiological activities. These differences are listed in Table 22.2.

Accumulated evidences indicate that auxins and gibberellins act both independently and together, depending upon the type of plant and the conditions under which the plant grows. The fact whether the auxins and the gibberellins interact or not is not conclusively proved.

CYTOKININS

DISCOVERY AND NOMENCLATURE

Auxins and gibberellins, besides inducing cell elongation, also do promote cell division under certain conditions. But this behaviour of them is an exception rather than a rule, However, there exist in plants many substances inducing cell division. For example, Van Overbeek et al (1941) found coconut milk as an active stimulant of cell division. Later, in 1955 Miller et al isolated a "cell-division-stimulating factor" from yeast DNA. It was named as kinetin because of its amazing power to stimulate cell division (cytokinesis) in the presence of an auxin. In subsequent years, many other compounds promoting cell division have been synthesized. Miller and his associates (1956) have grouped all such compounds including kinetin under a generic name kinin. Letham (1963) proposed the term cytokinins for such substances. This term is the most acceptable one.

Fairley and Kilgour (1966), however, prefer to use the term 'phytokinins' for such substances in order to distinguish them from the peptide hormones of animal gastrointestinal tract.

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DEFINITION

Skoog, Strong and Miller (1965) have defined cytokinins as chemicals which, regardless of their activities, promote cytokinesis (cell division) in cells of various plant organs.

Fox (1969) has defined cytokinins as chemicals composed of one hydrophilic adenine group of high specificity and one lipophilic group without specificity.

OCCURRENCE

Although kinetin does not occur in nature but other kinins are found occurring widely in plants. The naturally-occurring kinins do not occur free in nature but are normally bound to a pentose sugar, ribose and sometimes to an inorganic phosphate, the ribonucleotide.

Fruits and endosperm are the richest sources of kinins. Coconut milk and corn endosperm possess the active substance. Substances with cytokinin activity have also been reported in tomato juice, in floral extracts of apples and pears and also in cambial tissues of certain plants. A kinetin-kike substance is also present in peach embryo (Powell and Pratt, 1964) and sunflower root exudate (Kende, 1964).

The cytokinins are synthesized mostly in roots and probably originate at the root tips. Whether the shoots also synthesize cytokinins or else receive their cytokinin requirement from the roots is not certain.

CHEMISTRY

Chemically, kinetin ($C_{10}H_9ON_5$) is 6-furfurylamino-purine. It is formed from deoxyadenosine which is a degradation product of DNA (Hall and de Ropp, 1955). The

Contd...P/13.

structural formulae of kinetin and its 3 structural analogues are given in Fig. . All these substances promote cell division.

Apart from the above-mentioned kinins, Letham (1963) successfully isolated a cytokinin in pur crystalline form immature maize seeds. It was named as zeatin (Fig.22.14) and identified as 6-(4-hydroxy 3-methylbut-trans-2-enyl) aminopurine. Zeatin is more powerful than any other known cytokinin probably because of the presence of a highly reactive allylic OH group in its side chain.

Fleissner and Borek (1962) have described compounds such as N^6-methylamino,purine and N^6, N^6-dimethylaminopurine. These are widespread in plants and have cell division stimulating property. Recently, a cytokinin called N^6-purine has been isolated from serine-transfer-RNA of yeast cells by Hall and others in 1966.

PHYSIOLOGICAL ROLES

Certain physiological process which are influenced by the cytokinins esp., kinetin are given below:

1. CELL DIVISION. Kinins are notable for their stimulatory effect on cell division. Using tobacco pith cultures Skoog and Miller (1957) found that, in addition to IAA, kinetin is also needed for growth. The growth response is much more pronounced when both IAA and kinetin are used together in right ratio of concentrations. When either of them is used alone, a little response is produced which is due to the presence of small amounts of endogenous kinetin-like substances and IAA, already present in the tissues.

The process of cell division completes in 3 steps, viz., DNA synthesis, mitosis and cytokinesis. Studying the

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specific influence of IAA and kinetin alone on any of these 3 steps, Patau, Das and Skoog (1957) found that IAA is involved in the first two steps of cell division (i.e., in DNA synthesis and mitosis) and that the last step (i.e., cytokinesis) is controlled by kinetin. It has been suggested that the adenine moiety of the kinetin molecule is essential for cell division.

2. CELL ELONGATION. Besides auxins and gibberellins, kinetin also promotes cell elongation. Such promotion after kinetin treatment has been observed in tobacco pith cultures (Glasziou, 1957), tobacco roots (Arora et al, 1959) and bean leaf tissues (Powell and Griffith, 1960). Since cell elongation induced by kinetin has been well established, the kinetin should not be regarded as exclusively a cell division factor.

3. ROOT GROWTH. Kinetin is capable of stimulating as well as inhibiting root development. Skoog and Miller (1957) found stimulatory effect of kinetin, when applied along with IAA, on root initiation and development in stem callus cultures. Similarly, kinetins also induced increase in dry weight and elongation of the roots of lupin seedlings (Fries, 1960).

4. SHOOT GROWTH. The callus tissue of tobacco can be kept in an undifferentiated state so long as the proper balance of IAA and kinetin is maintained. If, however, the amount of kinetin is increased, leafy shoots are initiated to develop. Bean seedlings, soaked in kinetin solution, also showed an increase in dry weight and a marked elongation of stem and petioles (Miller, 1956).

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5. MORPHOGENESIS. Cytokinins can cause the formation of organs in a variety of tissue cultures. For instance, Skoog and Miller (1957) observed that tobacco pith callus can be made to develop either buds or roots by changing the relative concentrations of kinetins and auxins. High kinetin and low auxin contents result in the production of buds. In reverse condition (high auxin and low kinetin), however, the roots appear on the pith.

The kinins also stimulate the production of buds in leaf segments of various plants such as Saintpaulia innantha, Bryophyllum sp and Begonia sp,

In addition to the root and shoot differentiation, the cytokinins also bring about other morphogenetic responses. These are :

(a) maturation of proplastids into plastids
(b) differentiation of tracheids
(c) induction of parthenocarpy
(d) induction of flowering

6. COUNTERACTION OF APICAL DOMINANCE. As discussed earlier (refer page 490), the auxins emanating from the apical bud inhibit the growth of lateral buds (apical dominance). Wickson and Thimann (1958) studied the antagonistic effect of auxin and kinetin in apical dominance using pea stem sections in culture solutions. They found, as might be normally expected, that the growth of lateral buds is inhibited when the culture medium contain IAA and is uninhibited when the culture medium does not contain IAA. They further noted that addition of kinetin, along with IAA, stimulates the growth of lateral buds.

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The above workers also conducted experiments with entire shoots, i.e., with the apcial bud intact. So long as the apical bud is present, the lateral buds do not develop but removal of the apical bud leads to the stimulation of growth of the lateral buds. If, however, the intact shoot is soaked in kinetin solution, the inhibition of lateral buds is checked to a great extent or in other words the lateral buds tend to develop, Although less vigorously, as if the apex of the shoot has been cut off. The above findings point out towards the possibility of controlling apical dominance by maintaining a proper balance of concentrations between IAA and the endogenous kinetin-like substances.

7. BREAKING DORMANCY OF SEEDS. Cytokinins are also effective in breaking seed dormancy in lettuce, tobacco, white clover and carpet grass. Thimann (1963) suggests that the site of cytokinin action in such cases is the cotyledon. Furthermore, the inhibitory effect of infra-red light on germination of lettuce seeds is also alleviated by kinetin treatment.

The seeds of parasites such a Striga asiatica require the presence of host plant for germination. But when treated with kinetin, the seeds germinate even in the absence of their host.

8. DELAY OF SENESCENCE (= Richmond-Lang effect).

The term senescence refers to the ageing of the leaves which is associated with the loss of chlorophyll and the breakdown of proteins. Richmond and Lang (1957) showed that the senescence in the detached leaves of Xanthium could be postponed for many days by kinetin treatment. This effect of kinetin in retarding senescence (or ageing) is known as

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Richmond-Lang effect. According to Mothes and Engelbracht (1961), the cytokinins have the ability to attract certain substances including auxins and to prevent the movement of leaf components out of the treated area. However, the mobilizing effect of cytokinin may actually induce senescence in other parts of the plant. Osborne (1962) suggests that the high protein content in kinetin treated areas is probably due to enhanced protein synthesis than their breakdown. The protein synthesis, in its turn, is dependent on RNA synthesis, a process governed by kinetins.

A correlation between the age of the leaf and the kinetins has been established. Mature leaves of tobacco respond more vigorously to kinetin treatment in delaying senescence than the young leaves.

9. ROLE IN ABSCISSION. Cytokinins can accelerate as well as retard the process of abscission in leaf petioles depending on the site of their application (Osborne and Moss, 1963). In explant petioles of Coleus blumei, Gupta and Kaushik (1969) reported accelerated abscission on kinetin application.

OTHER NATURAL GROWTH HORMONES IN PLANTS

1. ETHYLENE

Although the presence of ethylene has been shown in certain fungi (Penicillium digitatum, Alternaria citri) and in the leaves, flowers and fruits of many higher plants since long, its recognition as a natural plant growth hormone has only recently been confirmed by Pratt and Goeschl in 1969. Ethylene also occurs in minute quantities in city gas and in tail gases of blast furnaces. It is a gas of peculiar odour and is sparingly soluble in water but a little more in ethanol and ether. It is inflammable and hence the ignition

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of a mixture of ethylene with air leads to explosion.

2. ABSCISIC ACID

The structure of Abscisic acid is given in Fig. Eagles and Wareing (1963) isolated an inhibitor from the birch leaves held under short day conditions. When this substance was reapplied to the leaves of birch seedlings, apical growth was completely arrested. As this substance induced dormancy, they named it as dormin. Later in 1965, Ohkuma et al isolated an inhibitor from cotton fruits and named it abscisin II. The same year, Cornforth and his associates isolated a growth inhibitor from sycamore and pointed out that both dormin and abscisin II are identical. Abscisin II is peculiar in that it is effective in much lower concentration than phenolic inhibitors and is accumulated under short day conditions.

Since its isolation and characterization, abscisin II has been found to perform a number of physiological functions:

1. It accelerates abscission and senescence in many plants such as cotton.
2. It inhibits seed germination in ash and lettuce.
3. It inhibits flower induction in Lolium sp.
4. It inhibits growth of IAA-induced oat coleoptiles.
5. It inhibits GA-induced enzyme synthesis in barley aleurone layers.
6. It inhibits completely the sprouting of potato buds.

Development of Mammals

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Introduction

Fertilisation brings about some change in the egg structure which undergoes cleavage. We come across diverse pattern of cleavage in the eggs of different animals, depending upon the amount of yolk it contains. Even in the three subdivisions of the class Mammalia we find there exist some difference in their mode of development.

a) Subclass Prototheria: Eggs have large amount of yolk, showing essentially reptilian pattern of development.
   Eg: Ornithorhynchus Echidna

b) Subclass Metatheria(the marsupials): The developing embryos receive nourishment from the mother in the uterus though this adaptation is not so well developed as in Eutheria. The yolk though present is ejected at the beginning of cleavage.

With the disappearance of yolk, mammalian eggs have reverted to complete cleavage, but subsequent development bears ample evidence of the former presence of yolk and in many respects the morphogenetic process resemble those in meroblastic eggs with a discoidal type of cleavage.

Cleavage

1. Soon after fertilisation cleavage starts.
2. Cleavage is complete, holoblastic blastomeres are more or less of equal size but the cleavage is not as regular as in oligolecithal eggs.
3. The first cleavage is vertical and divides the blastomeres into two unequal blastomeres.
4. Synchronization of the mitosis in the blastomeres is lost very early.
5. Second cleavage is also vertical but at right angles to the first cleavage. One of the blastomeres divides into two resulting in the occurrence of 3 blastomeres.
6. The third cleavage is horizontal.
7. Subsequently five, six and seven blastomeres and so forth are formed.

Speed of Cleavage

8. Overall spreed of the cleavage is much lower than in many other animals. Several hours elapse between successive divisions.

Formation of morula

9. As a result of cleavage a solid mass of cells, a morula, in which some cells (inner mass of cells) are large and lie inside completely cut off from the surface by a enveloping cells, which are smaller.

10. In due course the superficial cells join to form a distinct epithelial layer.

11. This layer gives rise to parts(the embryonic membra and serves to

    a) attach the embryo to the uterine wall.

    b) mediates in the supply of nourishment to the embryo from the maternal body via the placenta.

12. The outer layer of the mammalian embryo is known as the trophoblast.

13. The cells lying in the interior are known as inner cell mass and it is these cells that provide material for the formation of the embryo proper. They are therefore be referred to as the formative cells.

14. The two kinds of cells are distinguishable rather early by certain physiological properties, which may be shown by using specific staining methods.

15. 'Cells of inner cell mass have been found(in the rat) to be more basophilic than trophoblastic cells, suggesting a higher content of cytoplasmic nucleic acids(Jones-Seaton,1950) and also been found to cont the enzyme alkaline phosphatase, which is lacking i trophoblastic cells (Dalaq,1954).

Formation of blastocoel

16. Soon a cavity appears inside the compact mass of cel of morula. The cavity is formed of crevices which ap between the inner cell mass and cells of the trophoblast.

17. Fluid is imbibed into this cavity, so it enlarges, whole embryo becomes bloated to the same degree.

18. The trophoblast becomes lifted off the inner cell ma on most of its inner surface, and it remains attache one side only,which corresponds later to the dorsal of the embryo.

19. The mammalian embryo at this stage is called blastoc

20. The cavity of blastocyst may be compared to the blastocoel, but the embryo as a whole differs essent from a blastula, since its cells are already differe ted into two types:

    a) the inner cell mass
    b) and the cells of the trophoblast

21. Role of trophoblast and inner mass of cells

    There is experimental evidence that the properties of inner cell mass and those of the tropholast are alrea distinctly different at this stage.

    Three and a half day-old mouse embryos were cut in pieces, so that some pieces contained only inner cell mass and other consisted of trophoblast cells (Garne 1972). It was found that clumps of inner mass of cel fused together in larger masses, while clumps of trophoblast cells formed vesicles which did not adhe to one another.

22. When introduced into uteri of pseudopregnant mice, inner cells mass did not react with the uterine wall thus they did not become implanted and thus did not develop further.

23. On the otherhand, the vesicles formed from the trophoblast, when introduced into the uteri, established connection with the uterine wall as in the development of normal embryos but later failed to proliferate. This experiment clearly reveals the roles of the two components of the early mammalian embryos.

Formation of the hypoblast

24. A layer of very flat cells appear on the interior surface of the inner cell mass, that is, on the surface facing the cavity. This layer of flat cells corresponds to the lower layer of cells of the chick blastoderm, the hypoblast. The cells respresent the presumptive endoderm or at least a part of it.

25. The origin of the hypoblast endodermal cells is a subject of some controversy. The generally accepted view is that the endodermal cells are split off from the inner cell mass.

26. There are indications, however, that the endodermal cells in some mammals such as the elephant shrew(Van der Horst, 1942), or even in all mammals (Dalcq, 1954) are derived from the cells of the trophoblast. Some of these, near the edge of the inner cell mass, migrate inward along the internal surface of the inner cell mass and arrange themselves in a continuous layer, spreads out later to enclose the cavity or the yolk sac.

27. Unfortunately, the method of local vital staining or marking cells could not as yet applied to study of the migration of cells, which give rise to hypoblast cells in mammals.

28. Thus, the conclusion must be reached on the grounds of difference in the staining reactions of the various cells.

a) do not have basophilic stain
b) negative reaction for alkaline phosphatase.

This suggest that hypoblast is derived from trophoblast.

29. The trophoblast corresponds in position to the chorion of the taller embryos of reptilese birds but there is a difference that chrion develops in conjunction with amnion.

30. The inner cell mass then spreads out and becomes arranged into a plate, resembling the epiblast of the blastodisc of reptiles and birds.

31. The arrangement of the formative cells in the mamamlian blastocyst is at this stage similar to that in the avian blastodisc prior to the apperance of primitive streak but under the blastodisc of mammal there is a fluid filled cavity.

32. In some mammals where the development is primitive (rabbit) the layer of trophoblast, Raubers layer, over the epiblast becomes temporarily superficial.

33. In higher mammals this layer never disappears and the formative cells are never exposed to the exterior.

34. The blastodisc consisting of epiblast and a hypoblast, becomes quite sharply delimited from the remainder of the embryo.

35. Epiblast: Consists of a thick plate of columnar cells clearly distinguishable from the flatter and more irregularly arranged cells of the trophoblast.

Hypoblast: Cells on the underside of the blastodisc may become cuboidal or even columnar and differ from extra embryonic endodermlining the internal surface of the trophoblast. At the edge of the blastodisc. This thickening is the prechordal plate which forms the roo of the archenteron and it denotes the anterior end of the embryo.

Gastrulation

36. A primitive streak is formed and a Hensen's node is seen at the anterior end of the primitive streak.

37. Primtive streak is shorter than birds and do not surpa half the length of the blastodisc, being confined to posterior part.

Formation of Endoderm

38. The cells of the primitive streak migrate downward an sideways between the epiblast and the hypoblast and th contribute to the formation of the endoderm (. and Streeter,1941).

Formation of mesoderm

39. The loose cells migrating sideways give rise to the la of mesoderm.

Formation of notochordal rudiment

40. The cells migrating forward from Hensen's node remain packed more closely and give rise to the "head process the notochordal rudiment.

Archenteric canal

The notochordal rudiment in some mmals (including man) is perforated by a canal starting from Hensen's node known as archenteric canal.

In other mmals, either there is no archenteric canal the canal, though present in the notochordal rudiment, does not open to the surface at Hensen's node.

Where an archenteric canal is present, its ventral wa fuses later with the hypoblast and is then perforated the archenteric cavity opens into the yolk sac cavity.

Subsequently, the notochord separates itself from the endoderm, and the endoderm closes underneath the noto- chord, forming again a continuous layer.

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THE MODERN SYNTHETIC THEORY OF EVOLUTION

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The modern, synthetic theory of evolution recognizes four basic types of processes: GENE MUTATION, CHANGES IN CHROMOSOME STRUCTURE AND NUMBER, GENETIC RECOMBINATION, and NATURAL SELECTION. Thefirst three provide the genetic variability without which change cannot take place; natural selection guides populations of organisms into adaptive channels (Figure 1.1.). In addition, three accessory processes affect the working of these four basic processes. MIGRATION of individuals from one population to another, as well as HYBRIDIZATION between races or closely related species both increase the amount of genetic variability available to a population. The effects of CHANCE, acting on small populations, may alter the way in which natural selection guides the course of evolution. The purpose of the present book is to review our knowledge of each of these processes, and to show how they are interrelated with each other. The more we know about the four basic processes, the less reason we have for believing that any other basic processes remain to be discovered. We do not need to search any more for hidden causes of evolution. Nevertheless, we do need to understand much more about the way in which known processes interact with each other.

At the outset we must recognize that at least in higher organisms, and perhaps in microorganisms as well, the three processes, mutation, genetic recombination, and natural selection, are equally indispensable for evolutionary change to take place. Speculations as to which of the three is the most important are completely pointless. The best way to understand their interrelationships is to recognize that all populations of sexually reproducing organisms contain a large "gene pool" of genetic variability. Like a natural pool of water, the gene pool maintains a dynamic equilibrium between inflow and outflow of genes, and may become larger or smaller, depending upon various external and internal factors. Genes may be added to the pool (1)by immigration from other gene pools, which requires crossing or hybridization between immigrants and old residents of the population; or (2) by mutation, followed by spread of the mutant allele through the population . Genes are removed from the pool chiefly by (1) natural selection, which constantly cleanses the pool of unfavourable mutations and builds up adaptive complexes of genes, and (2) chance elimination of alleles, which takes place in small populations or during reductions of population size. Genetic recombination, following the principles of Mendelian heredity, is constantly reshuffling the genes in the pool, presenting new combinations for acceptance or rejection by natural selection. Its importance lies in the fact that adaptiveness of an individual rarely if ever depends upon the independent action of individual genes or gene mutations. Due to constant interaction between genes at different loci, or EPISTASIS, the adaptive value of most genes that are retained in populations depends upon their ability to form favourable combinations with other genes.

Natural selection, which results from interactions between populations and their environment, may either stabilize gene composition by eliminating most or all immigrants and mutants, or change it in various ways. Evolution takes place through alterations of the frequency of genes and gene combinations in the population, brought about by natural selection. Finally, reproductive isolation, which includes all the barriers to gene exchange between populations, has a canalizing effect. Since the richness and organizational complexity of the gene pool make possible several different responses to the same kind of environmental change, populations that are reproductively isolated from each other are almost certain to evolve in different directions, while those that are not so isolated because of gene exchange, will evolve in the same direction.

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ORIGIN OF SPECIES

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The medium of evolution is the population, a geographically localized aggregation of members of a given species. The raw materials of the evolutionary process are the inheritable variations which appear among the individuals of such a population. And the mechanism of evolution may be described as natural selection acting on the inheritable variations of a population.

In a population, the members interbreed preferentially with one another and they also interbreed occasionally with members of neighbouring sister populations (see Chap.8). The result of the close sexual communication within a population is a free flow of genes. Hereditary material present in a part of a population may in time spread to the whole population, through the gene-pooling and gene-combining effect of sex. Therefore, in the course of successive sexual generations, the total genetic content of a population may become shuffled and reshuffled thoroughly. We may say that a population possesses a given gene pool and that the interbreeding members of the population have free access to all components of that pool. Moreover, inas much as sister populations are in occasional reproductive contact, the gene pool of one population is connected also to the gene pools of sister populations. In this way, the total genetic content of an entire species continues to be

31.1 Concept of a gene pool. In a species, genes flow within and between populations. The total gene content of the species thus represents a gene pool to which all members of the species have access. Genes normally cannot flow between the gene pools of two different species, shuffled about among the member organisms (Fig.31.1).

Evolution operates via the gene pools of populations. We already know from Chap.30 how changes in genetic systems, hence inheritable variations, may arise: by sexual recombination and by mutation. In each generation, some individuals may appear exhibiting new trait variations, as a result of either recombinational or mutational process (see, for example, Fig.8.1). If these variant organisms survive and have offspring of their own, then their particular (mutational) genetic innovations will persist inthe gene pool of the population. In the course of successive generations, the genetic novelty may spread to many or all members of the population.

Whether or not such spreading actually takes place depends on natural selection. This term is synonymous with differential reproduction. Either "natural selection" or "differential reproduction" means simply that some individuals of a population have more offspring than others. Clearly, those leaving more offspring will contribute a proportionately greater percentage of genes to the gene pool of the next generation than those leaving fewer offspring. If, therefore, differential reproduction continues in the same manner over many generations, the abundant reproducers will contribute a progressively larger number of individuals to the whole population.

As a result, their genes will become preponderant in the gene pool of the population (Fig. 31.2).

Which individuals leave more offspring than others? Usually, but by no mans necessarily, those that are best adapted to the environment. Being well adapted, such individuals on the whole are healtheir and better fed, may find mates more readily, and may care for their offspring appropriately. However, circumstances may on occasion be such that comparatively poorly adapted individuals have the most offspring. Instances of this are sometimes encountered in human populations, for example. In any event, what counts most in evolution is not how well or how poorly an organism copes with its environment, but how many offspring it manages to leave. The more there are, the greater a role will the parental genes play in the total genetic content of the population. By and large, the well-adapted organism contributes most to the gene pool.

Therefore, if an inheritable variation appears in an organism and if, through differential reproduction in successive generations, the progeny of that organism becomes numerically more and more abundant, then a given genetic novelty will spread rapidly throughout the population. As a result, a trait variation originating in one organism will have become a standard feature of the population as a whole.

This is the unit of evolutionary change. Many such unit changes must accumulate in a population before the organisms are sufficiently altered in structure or function to be established as a new species.

All evolution operates through the basic process just descsribed. In brief, it consists of:

1. appearance of inheritable variations by sexual recombination and mutation

2. spreading of these variations through a population by differential reproduction in successive generations.

In as much as inheritable variations originate at random, evolutionary innovations similarly appear at random. But in as much as the best reproducers are generally the best adapted, evolution as a whole is directed by adaptation and is oriented toward continued or improved adaptation. It is therefore not a random process.

Note that, in this modern view of evolution, natural selection is fundamentally a creative force; for its important effect is to spread genetic novelty, hence new traits, through a population. It is also a peaceful force, involving reproduction, not "struggle for existence" or "survival of the fittest." Organisms actually struggle rather rarely. Indeed, animals try to avoid struggle and attempt to pursue life as inconspicuously as possible, eating when they can, reproducing when they can. And plants have never been seen to engage in struggles at all. Moreover, natural selection does not "eliminate the unfit". The "fit" may be the mightiest and grandest organism in the population, but it might happen to be sterile. And the "unfit" could be a sickly weakling, yet have numerous offspring. The point is that neither "survival" nor "elimination" is actually at issue. The only issue of consequence here is comparative reproductive success. Indirectly, to be sure, health, fitness, and even actual physical struggles may affect the reproductive success of organisms. To that extent such factors can have evolutionary consequences. But what in Darwin's day was regarded as the whole of natural selection is now clearly recognized to have only a limited, indirect effect on evolution. The whole of natural selection, directly and indirectly, undoubtedly is differential reproduction.

THE GENETIC BASIS

The Hardy-Weinberg Law

From the preeeding, we may conclude that evolution is characterized by a progressive change of gene frequencies. Thus, inthe course of successive generations, the proportion of some genes in the population increases and the proportion of others decreases. For example, a mutation mau at first be represented by a single gene, but if by natural selection this mutation spreads to more and more individuals, then its frequency increases whereas the frequency of the original unmutated gene decreases. Clearly, the rates with which gene frequencies change will be a measure of the speed of evolution. What determines such rates?

Suppose we consider a large population in which two alleles, A and a, occur in certain frequencies. In such a population, three kinds of individuals will be found, namely, AA, Aa, and aa. Let us assume that the numerical proportions happen to be

AA	Aa	aa
36%	48%	16%

Assuming further that the choice of sexual mates is entirely random, that all individuals produce roughly equal numbers of gametes, and that the genes A and a do not mutate, we may then ask how the frequency of the genes A and a will change from one generation to the next.

Since AA individuals make up 36 percent of the total population, they will contribute approximately 36 percent of all the gametes formed in the population. These gametes will all contain one A gene. Similarly, aa individuals will produce 16 percent of all gametes in the population, and each will contain one a gene. The gametes of Aa individuals will be of two types, A and a, in equal numbers. Since their total amounts to 48 pe cent, 24 percent will be A and 24 percent will be a. The overall gamete output of the population will therefore be

parents	gametes	parents	gametes
36% AA	36% A	16% aa	16% a
48% Aa	24% A	48% Aa	24% a
	60% A		40% a

Fertilization now occurs in four possible ways: two A gametes join; two a gametes join; an A sperm joins an a egg; and an a sperm joins an A egg. Each of these possibilities will occur with a frequency dictated by the relative abundance of the A and a gametes. There are 60 percent A gametes. Accordingly, A will join A in 60 percent of 60 percent of the cases, that is, 60 X 60, or 36 percent of the time. Similarly, A sperms will join a eggs in 60 X 40, or 24 percent of the cases. The total result:

Sperms	Eggs		Offspring
A	A	60 X 60	36% AA
A	a	60 X 40	24% Aa
a	A	40 X 60	24% Aa
a	a	40 X 40	16% aa

We note that the new generation in our example population will consist of 36 percent AA, 48 percent Aa, and 16 percent aa individuals. These are precisely the same proportions we started with originally. Evidently, gene frequencies have not changed.

It can be shown that such a result is obtained regardless of the numbers and the types of gene pairs considered simultaneously. The important conclusion is that, if mating is random, if mutations do not occur, and if the population is large, then gene frequencies in a population remain constant from generation to generation. This generalization is known as the Hardy-Weinberg law. It has somewhat the same central significance to the theory of evolution as Mendel's laws have to the theory of heredity (Fig.31.3).

The Hardy-Weinberg law indicates that, when a population is in genetic equilibrium, that is, when gene frequencies do not change, the rate of evolution is zero. Genes then continue to be reshuffled by sexual recombination and, as a result, individual variations continue to originate from this source. But the overall gene frequencies do not change.

Of themselves, therefore, the variations are not being propagated differentially. Evolution consequently does not occur.

What does make evolution occur are deviations from the "ifs" specified in the Hardy-Weinberg law. Thus, mating is decidedly not random whenever natural selection takes place; genes actually do mutate; and populations are not always large. Singly and in combination, these three factors may disturb the genetic equilibrium of a population and may produce evolutionary change.

SPECIATION

The key process to be explained is how unit evolutionary changes in a population eventually culminate in the establishment of new species and higher taxonomic categories. As already shwon in Chap.8, a species may be defined as a collection of populations within which reproductive communication is maintained by interbreeding. We may now define a species alternatively as a group of populations sharing the same gene pool (see Fig.31.1). Within the pool a free flow of genes is maintained, but genetic flow between two such pools does not occur; a reproductive barrier isolates one species from another. The problem of speciation, therefore, is to show how reproductive barriers arise.

Geographic barriers between sister populations usually develop before biological reproductive barriers come into existence. Among geographic barriers, distance is probably the most effective. Suppose that, in the course of many generations, the populations of a given species grow in size and number and that, as a result of the increasing population pressure, the organisms radiate into a progressively larger territory. In time, two populations A and Z at opposite ends of the territory may be too far apart to permit direct interbreeding of their members. Although gene flow still takes place via the interconnecting populations between A and Z, individuals of A and Z no longer come into reproductive contact directly (Fig.31.7).

It is then almost certain that, by chance, different genetic innovations arise in A and Z and that different ones will be propagated within A and Z by natural selection. Such an effect will be particularly pronounced if the environments of A and Z are or become more or less different. If now the evolutionary changes within A and within Z occur faster than the speed of genetic flow between A and Z, then A and Z will actually become progressively different in structure or function. These two populations thus may come to represent two distinct subspecies (Fig. 31.8).

Geographic isolation here has set thestage for the development of initial differences between members of A and Z. If the differences accumulate, they may eventually become so great that gene flow between A and Z will stop altogether. For example, population A (or Z) may undergo a change in the reproductive organs such that mating with neighbouring populations becomes mechanically impossible. Or the protein specificities of A may so change that the gametes become incompatible with those of neighboring populations. Or the time of the annual breeding season in A may become advanced or delayed relative to that of neighboring populations. Or the individuals of A may become changed psychologically, so that they no longer accept mates from neighboring populations. Biological barriers of this sort will interrupt all gene flow between A and Z. These subspecies, isolated reproductively, then in effect will have become two different species (Fig.31.9).

Although an initial isolation due to distance is probably the most common kind, other forms of geographic isolation are encountered as well. The development of terrestrial islands surrounded by water or of aquatic islands surrounded by land, the interposition of a forest belt across a prairie or of a prairie belt across a forest, the appearance of mountain barriers, river barriers, temperature barriers, or of many another physical barrier, each may result in geographic isolation. With reproductive contact then lost between two populations, evolution in each may henceforth follow entirely different courses. In effect, the parental species will become split into two new ones. At first, the descendant species will still be rather similar structurally and functionally. In time, however, evolutionary changes are likely to introduce progressively pronounced differences, including biological barriers to interbreeding. These add to and reinforce the environmental ones already in existence.

In two just-formed sister species, interbreeding often may still take place if the isolating condition is removed, but in nature such removals do not normally occur. Therefore, when two different species do not interbreed in nature, this does not always mean that they cannot interbreed. In many cases, members of different species may be brought together in the laboratory and there they interbreed perfectly well. For example, swordtails and platys, two species of tropical fish (Fig.31.10), may under certain conditions have offspring inthe laboratory. But in nature they almost never do because they are isolated reproductively; although they live together in the same rivers, biological barriers discourage crossbreeding. And after two sister species have been separated for long periods, interbreeding will no longer be possible even if members of the two are brought together artificially. Biological differences sooner or later become sufficiently pronounced to preclude interbreeding.

Speciation by this means is the principal way in which new species evolve. Such a process takes, on an average, about I million years. Consciously or unconsciously making use of this principle of reproductive isolation, man has been and is now contributing to the evolution of many other organisms. Here may be found direct proof that evolution actually occurs and, indeed, that it operates according to the mechanism described above.

The most ancient evolution-directigg effort of man is his successful domestication of various plants and animals. Darwin was the first to recognize the theoretical significance of domestication, and it was this , actually, which led him to his concept of natural selection. He reasoned that if man, by artificial selection and isolation, can transform wild varieties of given plants and animals into domesticated varieties, then perhaps natural selection and isolation, acting for far longer periods, can bring about even greater evolutionary transformations in nature. We know now that the domesticating process in fact does involve all the elements of natural evolution: first, deliberate physical, hence reproductive and genetic, isolation of a wild population by man; and second, long-continued, carefully controlled, differential reproduction of individuals "adapted" to human desires, that is, of individuals exhibiting traits considered desirable by man. The result is the creation of new strains, races, subspecies, and even species (Fig.31.11).

Furthermore, during the last few decades, rather rapid, man-directed evolution has taken place among certain viruses, bacteria, insects, various parasites, and other pest organisms. These live now in an environment in which antibiotics and numerous pest-killing drugs have become distinct hazards. And the organisms have evolved and are still evolving increasing resistance to such drugs. Indeed, the very rapid evolution of viruses and bacteria becomes a problem in research; laboratory populations of micro-organisms may evolve resistance to a drug even while the drug is being tested. Because micro-organisms have exceedingly short generation times, because their populations are physically small, compact, and easily reared, and because high mutation rates may be induced readily by X rays, they have become favorite test objects in evolution experiments.

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PLANT SPECIES

Dr. G. V. Gopal

ORIGIN OF NEW SPECIES

A species is not a static unit. Through time, it gets modified into new forms (Fig. 4.1). New species arise either by the slow transformation of existing species or existing species populations, or abruptly as a result of hybridization and polyploidy or through special evolutionary mechanism called catastrophic selection or even through counterfeit hybridization.

1. GRADUAL SPECIATION :

(a) Phyletic speciation. A species with restricted distribution may get gradually transformed into new species by the development of new genetic material produced by mutations and by continual incorporation of favourable genetic changes in its gene pool. The net result is that in place of one old species there is one new species. This process of gradual transformation of an old species into a new one is known as phyletic speciation.

(b) True speciation. In true speciation physical or biological isolation develops in a widespread species and in due course of time due to development of new acceptable genetic material and its continual incorporation there takes place slow divergence of isolated populations into two or more species (Fig. 4.2).

It was Mortiz Wagner who put forth a hypothesis of true speciation in 1868 which is known as geographical theory of speciation or even Allopatric theory of speciation. According to this hypothesis the first step in true speciation is the separation of original gene pool into two or more parts (Shrinkage) as a result of reproductive isolation brought about by changes in the environment or may be the consequence of long-range dispersal in which case, as has been emphasized by Stebbins (1960), self-pollinating species have a greater chance of successful establishment. The resulting gene pools in a bid

Contd...P/2.

to adjust themselves to their surroundings evolve in different directions. Gradually they become morphologically and physiologically quite different. This process is known as differentiation As a result of reproductive isolation and differentiation the fragmented parts of the original gene pool come to occupy a definite geographic range. Sometimes as a consequence of further environmental changes an expansion of this range may take place resulting in overlapping. The overlapping may also result from migration of different populations into a previously unoccupied area (Fig. 4.2). It is quite possible that the two populations that happen to come together due to overlapping might have already differentiated physiologically and genetically to a degree that they are unable to form viable zygotes. In such an instance speciation process has already been completed without interaction. Conversely, the two populations may have almost similar ecological requirements and potentiality for hybridization. In that case, they will compete with each other. One population may outcompete the other restoring the original non-overlap range. Or, the two populations, as a result of further selection, may divide the environment in a way that competition is wanting and simultaneously some sort of breeding barrier is established restricting the formation of hybrids. Or, in an overlapping range/area two populations may hybridize to form one interfertile population. In fact, whenever there is competition between two populations they always tend to displace each other, that is, requirements of one or both populations change in a way that reduce competition. It has aptly been put forth by F.G. Gause (1934) in his 'Exclusion principle' which states, "No two forms can share exactly the same environmental requirements for an indefinite period of time, eventually one form will replace the other."

Thus, the two most important steps in the process of true speciation are reproductive isolation and differentiation. Sometimes secondary merger does take place but it is not an essential step of the process.

2. ABRUPT SPECIATION

Abrupt speciation usually occurs either as a result of polyploidy, catastrophic selection or counterfeit hybridization.

Contd...P/3.

POLYPLOIDY

This process results in the multiplication of genome or chromosome number of a plant. It has been observed that mostly resultant polyploid individuals are incapable of forming fertile offspring with individuals of the parental stock. The resultant polyploid individuals thus acquire instant reproductive isolation and if these are able to exploit an ecological situation to their benefit, the result is the formation of a new species. W.H. Lewis, (1967), however, asserted that a kind of ecological requirement need not follow chromosome doubling. This generalization was based on his observations that 2x and 4x cytotypes of Claytonia virginica, Hedyotis purpurea, H. longifolia and Oldenlandia capensis could grow only a few meters apart on ecologically similar niches. This process indeed has played a major role in the evolution of angiosperms where about 40% of the total species are polyploid. Four main types of polyploids recognized are autopolyploids, true or genomic allopolyploids, segmental allopolyploids and autoallopolyploids (Fig. 4.3) (cf. Stebbins, 1960). A large number of taxa once regarded to be autopolyploids are suspected to be of hybrid origin. Autopolyploids, in general, look remarkably similar to their diploid progenitors. Segmental allopolyploids simulate autopolyploids in close morphological resemblance to diploid forms and in displaying of multivalent configuration at meiosis, and are mostly confused with them. Nothing can be said with certainty regarding their actual ancestry until both of its parents have been identified through hybridization experiments. Segmental allopolyploids possess the ability for genetic segregation with regard to morphological differences and also chromosomal differences forming sterility barrier between the parental species. Further, segmental alloploidy is an unstable condition which, guided by selection for fertility, will either evolve in the direction of auto or typical allopolyploidy. If chromosomes of the parental species were largely similar and differed by one or two small non-homologous segments, selection would tend to eliminate these non-homologous segments and favour evolution in the direction of autopolyploidy. If the situation

Contd...P/4.

was other way round, that is, chromosomes differing initially by numerous and large segments then selection would favour further differentiation of such chromosomes through mutations and chromosomal rearrangements and elimination of multivalent formation, thus, directing them towards typical allopolyploidy. Segmental allopolyploids sometimes form partly fertile hybrids through backcrossing with autopolyploid derivatives from either of their parental species. Typical allopolyploids usually stand out as clearly marked species as hybrids resulting from their backcrossing to either diploid parent or to their autopolyploid derivatives are usually partly and sometimes completely sterile. Apart from being fully fertile allopolyploids are strongly isolated from, and also morphologically discontinuous with their closest relatives. Thus, polyploid species complexes which pose difficult problems to systematists contain either segmental allopolyploids or autopolyploids or both. Autoallopolyploids exist only at the level of hexaploidy or higher. A very strong resemblance may result between an autoallopolyploid and one of its diploid ancestors if it contains two or more genomes derived from one species and only one genome from the other.

CATASTROPHIC SELECTION :

In 1962 Harlan Lewis proposed a special evolutionary mechanism which does not involve the process of polyploidy and leads to rapid speciation resulting from genetic isolation with little or no morphological differentiation. He observed that three species of Clarkia (Onagraceae) e.g C. franciscana, C. rubicunda and C. amoena were morphologically quite similar (Lewis and Raven, 1985 a, b). They crossed these species in all combinations to observe the fertility of hybrids and to note the differences in chromosome architecture as exhibited by chromosome pairing at meiosis. In every case nearly 2% of the pollen were found to be fertile and chromosome pairing in hybrids revealed that all the species differed drastically from each other in arrangement of the homologous segments. C. franciscana differed from C. rubicunda by at least three large translocations and four paracentric inversions and from

Contd...P/5.

C. amoena by at least two translocations and two or more paracentric inversions. The species C. amoena differed from C. rubicunda by three translocations and two inversions. Thus, the three species of Clarkia, which were morphologically very similar, were found to be dissimilar in respect of arrangement of genes on the chromosomes. The genetic isolation results from this dissimilarity. How such chromosomal alterations arise ? To explain this Lewis put forth the hypothesis of catastrophic selection according to which under certain unknown conditions (environment stress such as drought etc.) drastic chromosomal rearrangements may take place in a plant and if such a plant becomes detached from its parent population and is capable of establishing a population of its own with new chromosomal rearrangements then it results in the formation of a population which is genetically isolated from the ancestral population from its very inception, leading to the formation of a new species. What are the actual forces responsible for such drastic changes in some and so little in other taxa is still a mystery ! Probably a major force resides in genetic system of each species which controls and determines the way a species reacts to the environmental changes.

COUNTERFEIT HYBRIDIZATION :

It also serves as an ideal isolating mechanism (de Wet et al., 1984). The process involves non-random transfer of genes or non-random incorporation of DNA fragments from a non-functional sperm of a taxon into specific position of the genome of parthenogenetically developing egg of another taxon. How all this happens is still not known. While conducting hybridization experiments, with Tripsacum serving as female parent and Zea as male parent, de Wet and coworkers noticed that in Tripsacum some of the maternal offsprings resulting from parthenogenetic development of non-reduced female gametophytes though contained 2n = 36 Tripsacum chromosomes, yet were hybrids rather than maternal in phenotype. Such hybrids were termed counterfeit hybrids. In tripsacum genome Zea material got incorporated without true fertilization between Tripsacum egg and Zea sperm. These hyprids differed

Contd.,.P/6.

from Tripsacum parent both in phenotype and cytological behaviour. They produce sterile offspring when crossed with their maternal Tripsacum parent. Tripsacum cundinamarce (2n = 36) is believed to be a counterfeit hybrid between T. dactyloides var. meridonal and Zea mays that served as the pollen parent.

SPECIATION IN VEGETATIVELY REPRODUCING PLANTS

In vegetatively reproducing plants which include most of the monocotyledonous taxa sexual reproduction is of secondary importance and very often even absent in them. In most of the members of Liliaceae and Amaryllidaceae flowers are formed but meiosis is irregular resulting in sterile pollen and no seed set. Does this mean that such taxa have reached a static step in evolution and have lost the capacity for speciation with loss of sexual reproduction ? In nature and even under cultivation we observe that majority of such taxa are capable of producing numerous new variants indicating that they must possess some effective means of speciation unrealated to sexual reproduction. In the somatic tissue of some of the varieties of Caladium bicolor (2n = 28), a member of the family Araceae, Sharma and Das (1954) observed some cells with either varying chromosome number and/or chromosome structure from the normal ones. Such variations were always found to be at random i.e. without any zonation. Variations in chromosome structure could arise as a result of chromosomal rearrangements including deletions, duplications, inversions and segmental interchanges; and variations in chromosome number through non-disjunction, somatic reduction and possibly through partial endomitosis. Further abnormal karyotype in one of the varieties was found to be normal karyotype of another variety. It was, therefore, suggested that if an abnormal nucleus with numerically or structurally altered karyotype happens to enter the growing apex which gives rise to a daughter shoot through vegetative means the new shoot would comprise cells with altered karyotype. Such a shoot on detachment from the parent plant would give rise to a completely new form. As sexual reproduction is absent, such abnormalities are not lost through gametic inviability

Contd...P/7.

(Sharma, 1956). But numerical alterations are sure to upset the nucleocytoplasm ratio which in turn is likely to affect the metabolism of the cell. It has been suggested that such an imbalance at individual cell level is rather advantageous to such plants and facilitates further irregularities leading to somatic mutation.

ECOLOGICAL PROPERTIES OF A SPECIES

If we study the performance of plants of a particular species against any single environmental gradient we always get similar graphs as depicted in Fig. 4.5.

Each species has its own range of tolerance. This represents the range within which individuals of a particular species are able to survive with reference to particular environmental gradient. Another point which emerges is that all plants of a species, which are being tested against an environmental gradient, do not grow with equal vigour in all parts of their tolerance range. Within central part of their range plants normally grow most vigorously decreasing progressively towards tolerance limits where it becomes zero. The portion of the tolerance range where vigour is greatest is termed ecological optimum of that species. Comparable responses will be observed if the same species is examined along other environmental gradients provided its individuals are sensitive to such variables. The separate responses will comprise a great variety of tolerance ranges, ecological optima and vigour within these. The sum of tolerances along all gradients to which a particular species is sensitive has been termed ecological amplitude of the species. In fact, each species has its own tolerance range, ecological optimum and its own ecological amplitude. More the ecological amplitude of a species, more successful it is in nature. For its survival, however genetic variability is imperative. This results from genetic recombination or continuous interbreeding within an interbreeding population. A species population may survive some new environmental stress if sufficient genetic variability exists within it to ensure that some gene combinations impart drought resistance. Genetically identical population, on the contrary, would be faced with extinction if the single gene combination present did not impart drought resistance.

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Plant Modification

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The factors for modification can be broadly classified in three major categories namely: i) Environmental , ii) Genetical iii) physiological modification or adaptation of plants due these three major components.

Environmental Factor: The Environment as such is very difficult to define shortly. Environment consists of Edaphic, climatic, hydrological, Biotic as such. According to Hutchinson,1951,1958 introduced the concept of ecological niche referring it to the totality of biotic~~a~~ and abiotic factors to which a given species is uniquely adapted.

Periodicity of season also plays a dominant role in the phneology of the plant, same species collected from two different localities is not alike because the ecological factor at both places is not same.

Light Factor

Light Duration: Photoperiodism is characterised by great ecological significance. It explains why many plants in tropics where light period is almost constantly 12 hours, flower throughout the year and like wise, why so few plants in temperate, latitudes leave this ~~characteristic~~ penomena. It is also apparent that carticspecies must be long-day plants. They rarely flower when further south. Species requiring high temperature and long days to mature are definitely limited in their northern range.

Light quality: The quality of light is modified by clouds and fog. Water vapours absorb infrared light and from water-surface blue-green rays are mostly reflected or transmitted so that bodies of water appear blue or greenish blue. Red rays induce a great development of tissues and cause the cells to elongate very much. Blue-violet rays on the other hand inhibit growth and keep the cells small. Red and blue light are the most effective in photosynthesis by green plants. UV-rays have injurious effect upon cells and plant growth and in total the plant modification leads to varieties.

Phytochemical variation due to UV radiation frequency/intensity

Ultraviolet radiations have injurious effect on plant growth and it is therefore unfortunate that most of the ultra-violet radiation is absorbed by the ozone layer in the upper part of the atmosphere. In alpine regions, the intensity of ultra-violet radiation is much higher than at sea-level and is responsibile for the development of ~~fxx~~ flavours and anthocyanins in alpine plants.

Precipitation:

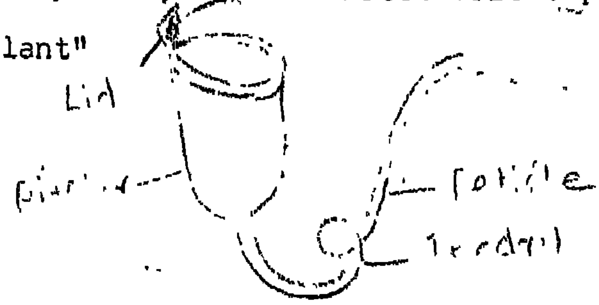
The principal forms of precipitation are rainfall, hail, snow and dew of these, rainfall is the most important as most plants absorb water from the soil. If the water level varies then the type of vegetation also varies as xerophyte ....

Mesophytes ..... and free floating hydrophyte ... submerged hydrophyte.

but some plants have modified in such way their organ structure to absorb this atmospheric water directly as in th case of Lichens, orchids, epiphytic plants. Valamens roots having valamentissue is developed in this group of plants.

Wind: Wind has both a direct and an indirect effect on plant life. The direct effect of strong winds is mechanical and consists in uprooting trees and breaking off branches and twig Strong winds also cause permanent curvatures in plants on ecposed places.

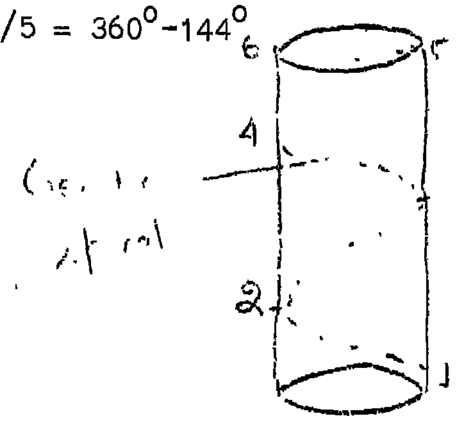
Altitude: Topographic factor, the ffect of altitude is best seen in mountains. The climate changes as we ascend, becoming progressively cooler. There is greater wind action greater altitudinal varaation leads the change in vegetation of that area. If the soil is deficient of N_2 - then also it leads to the production or gives an opportunity to the development of or community of plants, as ex: Insectivorous plants Nepethces "Pitcher plant"



Light ~~has~~ as factor has many other effect on plant modification as photosynthesis is the most important biological activity. Phyllotaxis is an important phenomenon in plants which occurs in systematic manner. Phyllotaxis means "spreading of leaves are otherwise" modern terminology says as Fibonacci series. They ca distichous due to some special types of chemical and particularly in case Psidum the plants exhibit mimicry Mi- [illegible].

i.e. 13, 57, 86, 42 i.e. 1/2 = 180^0

or 1,4,7,10,2,8,9,6,5,4,3 i.e. 2/5 = 360^0-144^0



Sometime the plant accumulate the alkaloids and some amino-acids like proline may act as defensive mechani sums is well known phenomenon.

In some case plant have modification of plant parts like spines throns, bristles, Pitcher, stingine hairs, Latex ect.

Genetical: In breeding, and out breeding the two different systems prevailing in higher plants are out crossing and inbreeding. In addition various asexual methods of reproduction have evolved in number of taxa. Outbreeding enhances recombination, heterozygosity, variability and evolutionary potential. Protandany and protosy are the two important floral-mechanisums which promote out breeding. Out breeding promoting systemx is called as heteromorphic system.

Self incompatibility: In majority of the higher plants pollen is incapable of germinating on stigmas or styles of the plant that produce it. Fertilisation of the egg is thus prevented. This condition is known as "Physiological or genetical self incompatibility". This can be early manipulated by using some temperature treatments, irradiation. Bud pollination, mentor effects, paraffin oil, organic solvents, carbondioxide.

Inbreeding systems: Inbreeding system ensure regular and optimal seed production even in the absence of pollinations and are of considerable importance to colonizing taxa in new habitats.

That there are variations in the photosynthesis process among green plants is well known. There are three variants namely, the C_2type(Calvin cycle) C_4 type (C_4 dicarboxylic cycl and CAM (Crassulacean Acid Metabolism). The lower groups and a great majority of flowering plants have C_2 type of photosynthe process. C_4 photosynthesis evolved as a response to changes in carbon and oxygen concentrations in the atmosphere during the course of plant evolution (Smith B.M. 1976). Instead of the usual phosphoglyceric acid, the initial product of the well kn Calvin-Benson Cycle (C_3), the C_4 plants fix carbon initially 4-carbon organic acid-oxaloacetic acid and then malic or aspar acid (Webster et al,1975). This type of photosynthesis is know occur in a large number of species in tropical and warm- temperate regions. A rough estimate put the number of such sp as 945 spread out in 196 genera and 18 families(Ramadas and Raghavan,1980). Such plants have been designated as C_4 plants Hatch and Slack (1970). They have highly specialised chlorench matous leaf bundle sheaths in which the chloroplasts hav slightly modified granar and wall structure. These anatomical specialisations are together known as "Kranz syndrome" and the species having them are called "Kranz species"(Tregunna et al Smith and Brown,1973).

Though restricted to a minority of angiospherm C_4 pathway exhibit interesting correlations and systematic implications. Smith and Brown (1973) have studied the Kranz syndrome in the Graminlae using carbon isotopes. In this fami is is confined to the three tropical subfamilies, Arundinoide Eragrostioideae and Panicoideae and holds promise in sorting the controversy over systematic positions of taxa like Aristi and Eragrostis(Rama Das and Raghavan,1980).

Within the genus Euphorbia, Crassulacean acid metbaolis has been detected in the subgenus Euphorbia and C_1-photosynth in subgenus Chamaesyce. Webster et al (1975) have concluded the different subgenera with different photosynthesis strate have completely independent origin and hence warrant the tre of the subgenus chamaesyce as a distinct genus.

In Centrospermae C_4 photosynthesis is known in seven o 11 families. The basal group phytolaccaceae, significantly C_2 photosynthesis(Marbry,1977). Other dicot families known C_4 genera are Boraginaceae, Compositae and Zygophyllaceae. C photosynthetic pathway is functionally and phylogenetically distinct from the other two. The CAm plants are relatively p synthetically inefficient but are efficient in conserving wa because their stomata open only at night (Mabry,1971). This type of photosynthesis is seen in members of families like Cactaceae, Aizoaceae and Euphorbiaceae.

Flavanoids have been one of the most exploited phytochemical character in relation to classification. Extensive surveys on the distribution patterns of these compounds have been carried out. Indeed, they will continue to be useful in classification at lower levels of taxonomic hierarchy, but such information on distribution alone would not be of great use in deducing phylogeny (Giannasi,1978b; Crawford,1978). Swain (1975) Harborne (1977) and many others have attempted to deduce phylogeny of these compounds. But, these speculations have mostly been based on their correlation with morphological or other nonchemical characters and hence has an element of circularity as Heywood (1973b) has pointed out.

MEDICINAL PLANTS

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The history of medicinal plants is intimately connected with the history of botany. Primitive man lived at the mercy of nature, in constant terror of diseases. From the earliest times, tribal priests and medicine men (witch doctors) used various plants, minerals and animal organs, usually in association with strange rituals and incantation, to drive out the evil spirits which they believed to be the cause of the disease. Astonishingly, these magical rites seemed to help . In some primitive tribes, a victim of disease was half-buried in soil for several days to exercise the malevolent spirits which had possessed him. Among the extremes of treatments was the chipping of holes in the skull to release the tormenting evil spirits. This theory of demoniacal possession lasted many centuries and exists even today in areas where people still live in primitive societies.

Records of early civilisation in all parts of the world reveal that a considerable number of drugs used in modern medicine were in use even in ancient times. The use of plants for curing various human ailments figured in ancient manuscripts such as The Bible, The Rig-Vedas, The Iliad and The Odyssey and the History of Herodotus. Over 6000 years ago, the ancient Chinese were using drug plants. The Egyptians, Babylonians, Sumerians, Greeks and Romans, all developed their respective characteristic Materia Medica. On the other side of the world, the

Aztecs, Mayans and Incas had all developed primitive medicine. Some of the ancient Egyptian text-books 'papyri' (such as the Edwin Smith Papyrus and the Ebers Papyrus), written as early as 1600 B.C. indicate that the Egyptians had an amazingly complex Materia Medica. Apart from the names of many medicinal plants then known, the papyri also included several hundred recipes or prescsriptions for various diseases. The Edwin Smith Papyrus (about 1750 B.C.) is now one of the prized collections of the New York Academy of Medicine.

In India, the ayurvedic system of medicine has been in use for over three thousand years. Charaka and Susruta, two of the earliest Indian authors had sufficient knowledge of the properties of the Indian medicinal plants. Their medical works the Charaka Samhita and the Susruta Samhita are esteemed even today as treasures of literature on indigenous medicine.

The Greeks and Romans were familiar with many of the present day drugs as is evident from the works of Hippocrates (460-370 B.C.), Aristotle (384-322 B,C.). Theophrastus (370-287 B.C.), Pliny the Elder (A.D.23-79), Dioscorides (A.D. 50-100) and Galan (A.D.131-201). They wrote extensively about medicinal herbs, giving their names along with a description of each plant, illustrations, their putative healing properties and also complex descriptions for the preparation of medicines. Hippocrates, the 'Father of Medicine', was the first to attempt a scientific explanation for diseases. His influence remains today in the Hippocratic oath taken by young doctors upon their graduation from medical school. Dioscorides' treatise on medicinal plants De Materia Medica remained the supreme authority for over sixteen centuries, during which the manuscript was laboriously copied and recopied with few additions. In like manner, the works of Aristotle, Galen and even Pliny were copied and handed down with increasing inaccuracies. During the Dark Ages (A.D. 400-1000) few new ideas were added. During the Middle Ages (A.D. 1000-1500) also, little significant botanical progress was made. Had biology progressed steadily from the time of Aristotle, there probably would have been no great plague and smallpox epidemics during the Middle Ages. One epidemic of the plague between 1347 and 1350 killed sixty million people in Europe, Asia and Africa.

About the beginning of sixteenth century, several herbals of considerable merit were published, such as those of Brunfels (1530), Bock (1539), Fuchs (1542), Cordus (1561) and L.Obel (1576). Although these works were a great improvement over earlier ones, they still propagated many myths and superstitions such as the 'Doctrine of Signatures' advocated by an eccentric genius Paracelsus (1493-1541), according to which all plants possessed certain signs given by God, which indicated their usefulness in treating diseases of similarly shaped organs in the human body.Plants, for example, with heart-shaped leaves were used for cardiac disorders; the sap of bloodroot (Sanguinaria canadensis L.) as a blood tonic; the walnut with numerous invaginations and convolutions for brain diseases and pomegranate seeds for dental diseases. Particularly striking was the myth of the mandrake plant (Mandragora officinarum L.) of the family Solanaceae. The fleshy and often forked roots of this plant somewhat resemble the torso and the lower limbs of a human figure and were believed to be quite efficacious for treating various human ailments. Since ancient Greek times, the resulting decoction of mandrake roots either prepared in boiling water or in wine, has had a reputation of producing sleep. It was perhaps the first true anaesthesia and later in 1889 was found to contain a mixture of pain-deadening alkaloids (podophyllin, mandragorin and hyoscine), the most important of these being hyoscine or l-scopolamine.

With the development of synthetic drugs, plant products lost their significance. In the last few decades, however, there has been probably more interest in drugs obtained from vegetable sources than at any time in history because of the success with the antibiotics, and other plant drugs such as rauwolfia (for the treatment of mental diseases), podophyllum (a cathartic, as well as for curing cancerous tumors in mice), aloe (a cathartic, as well as for the treatment of atomic radiation burn); veratrum (hypertensive agent), peyote (psychoactive drug) and sundry others. Sapogenins (closely related to steroids), obtained from many members of Dioscoreaceae and Agavaceae, can be converted into cortisone, into male hormone (testosterone) and female hormones (estrogens and progesterone). Sapogenins have many potential uses in the treatment of rheumatoid arthritis and the female hormones are used in contraceptive pills.

From the crude beginning of the earlier physician-botanists, the study of drugs and drug plants has developed into modern pharmacognosy (pharmacon = drugs and gnosis = to know) which deals with the knowledge of history, botany (including properties and methods of preparation of drugs), preservation and commerce of crude drugs. Pharmacology is the study of the action of drugs on an organ or organism. Nature has provided a rich storehouse of herbal remedies to cure all mankind's ills. Throughout the world primitive peoples have utilised several thousands of different plants and plant products as cures for human ailments. Many of them have been rendered obsolete today because of the synthesis of their active principles. Some are widely cultivated, but most are gathered entirely in the wild state.

The information on drugs and drug plants whose efficacy in medicine has been established is available in various authentic books known as 'pharmacopoeia' and the drugs included therein are described as 'official'. The most important of these pharmacopoeia are the 'United States Pharmacopoeia'. British Pharmaceutical Codex'; 'Indian Pharmaceutical Codex'; and 'National Formulary'. These works are constantly being revised and kept up-to-date.

The medicinal value of drugs is due to the presence of certain substances such as alkaloids, glycosides, resins, volatile oils, gums, tannins, etc. Some of these are powerful poisons if administered indiscriminately, while others are dangerously habit-forming. Even the most dangerous drugs can be of value to human beings, if judiciously employed. The danger of self medication is serious and extensive. People who believe themselves ill or simply off-colour physically or mentally, often use wonder-working drugs (happy pills) to relieve themselves from the tensions of modern living. These tranquillisers have proved so effective that their use has increased amazingly and has now outstripped all other drugs with the exception of the antibiotics. However, when used without proper medical advice, they may bring misery to millions.

The active principles of plant drugs are commonly more concentrated in storage organs. Roots, seeds, bark and leaves are much represented in the Materia Medica, flowers are less commonly used. While woods and woody parts of herbaceous stems are usually relatively inert.

Botanical drugs have been variously classified dependigg upon; the plant and plant parts from which they are derived, the disease for which they are used and their chemical nature.

We shall discuss below only some of the most outstanding drugs; other commercial drugs and drug plants will only be listed.

Atropa belladonna L. (n = 36)	Belladonna,
Family: Solanaceae	deadly nightshade

Belladonna has been extensively used in European medicine since the earliest times and is still regarded as one of the few indispensable drugs of plant origin. Because of the mydriatic action of the leaf juice when introduced into the eyes, the plant was frequently used by Italian and Spanish ladies as means of imparting a seductive appearance to their eyes.

The deadly nightshade is a native of Central and Southern Europe and Asia Minor. Nowea-days it is extensively cultivated as a medicinal crop in the United States. Europe and India. In India , it is found under cultivation chiefly in Kashmir and Chakrata (Uttar Pradesh). The plant which grows in the Himalayas and is also cultivated in Kashmir is not A. belladonna, but A.acuminata Royle differing in the leaf shape and the colour of flowers.

A. belladonna is a herbaceous perennial with a creeping rootstock, growing to a height of about 90-120cm and possessing alternately arranged ovate leaves and bell-shaped purplish flowers (yellow in Indian belladonna. A.acuminata). It bears shining brownish or black berries (Figure 15.1A).

The plants are raised from seeds sown in warm, shady places, not exposed to direct sunlight. Vegetative propagation through splitting of old rootstocks can be also practised. The soil should be a light, calcareous, well-drained loam containing decomposed humus and good quantities of minerals like potash and soda.

The leaves and tops are collected during the flowering season, when the alkaloidal concentration is greatest, varying from 0.9 to 1.23 per cent. The leaves (and fleshy roots, if used) are thoroughly dried, preferably with artificial heat for a few days and then pulverised.

All parts of the plant contain alkaloids but are more abundant in the physiologically active cells. A large number of alkaloids have been isolated which are collectively referred to as 'belladonna alkaloids'.

Atropine ($C_{17}H_{23}O_2N$), its isomer hyoscyamine and scopolamine ($C_{17}H_{21}O_4N$) are the three most used in medicines. Other related alkaloids, such as apoatropine, belladonine, noratropine, norhyoscyamine, tropacocaine and meteloidine are relatively unimportant therapeutically.

Atropine and hyoscyamine act as stimulants to the sympathetic nervous system and are employed as an antidote to opium. These are also used to reduce nasal secretions, sweating and also to control excessive salivation. Atropine has a stimulatory effect on the circulatory and respiratory systems. Internally, belladonna is used for the treatment of whooping cough and asthma and externally as a liniment to relieve neuralgic pain. Atropine is employed for dilation of the eye pupil during ophthalmologic examination and also to counteract muscle spasm (excessive muscular contraction).

Scopolamine, on the other hand, markedly depresses the parasympathetic nervous system thereby acting as a sedative or 'anti-insomniac'. It was formerly used with morphine to induce 'twilight sleep'.

The fruits are so poisonous that ingestion of three berries is sufficient to kill a child.

Besides *A.belladonna*, the leaves and seeds of other solanaceous plants such as jimson weed (*Datura stramonium* L.) and the leaves of the henbane plant (*Hyoscyamus niger* L.) contain atropine, hyoscyamine and scopolamine in varying proportions (Figures 15.1B-D). These solanaceous narcotics produce an intoxicating effect, when smoked or eaten, but continued used leads to disorders of the brain.

Cinchona spp. L.(x = 17)   Fever bark tree
Family: Rubiaceae   or quinine tree

The antimalarial property of Cinchona bark was known to the South American Indians from early times. Many stories are told regarding the discovery of *Cinchona*, revolving round the Countess of Chinchon, wife of the Viceroy of Peru, who was supposedly cured of malaria in 1638, after all other cures had failed, It is also learnt that the secret of the efficacy of this bark was revealed by a native maid out of affection for her mistress. The Countess was so pleased with the efficacy of the drug that she introduced it into Europe in 1640. In the eighteenth century, Linnaeus named it *Cinchona* in honour of this gracious lady. This often-quoted incident is, however, considered to be a myth. It is certain that Jesuit missionaries first brought it to Europe and its pharmaceutical value received recognition a few centuries later. The fame of the new remedy for malaria soon spread to other countries and the Peruvian bark was in great demand. For over two centuries (from the mid-seventeenth to the mid-nineteenth century), South America had a virtual monopoly of production. But due to reckless felling of the trees, it faced extinction that depleted the supplies in the Andean highlands.

Expeditions to collect seeds were sent out to the South American forests by the Dutch in 1852 and by the British government in 1859, in order to establish Cinchona plantations in South east Asia. In spite of strong vigilance of the forests by the native Indians, members of both the expeditions managed to smuggle seeds and seedlings out of South America. The seeds collected by the British expedition (under the leadership of Sir Clements Markham) became the basis of *Cinchona succirubra* Pav. plantations in India. Around 1861, Charles Ledger, an English resident in Bolivia, sent seeds of *Cinchona* to Europe. These seeds were collected by his faithful servant, who later died because of inhuman treatment by his compatriots for revealing the secret. Half of these seeds were taken by a planter to Ceylon and the remainder went to the Dutch Government. From Ledger's seeds, the Dutch established great plantations of *C.ledgeriana* Moen, in Java where climatic and topographical conditions were similar. Nearly 95 per cent of the world's export trade in quinine remained in the hands of the Dutch. All phases of *Cinchona* production, i.e. culture, harvesting processing and breeding were worked out, particularly in Java.

The Dutch venture flourished on a large scale until the outbreak of World War II. The capture of Java by the Japanese came as a serious blow to the United States and her allies. During the war, attention was again paid to the once rich South American jungles that were systematically exploited by North American technical expeditions. Several new sources were discovered, including the high yielding C.pitayensis Wedd. Latin America (especially Ecuador and Peru)became the chief producer between 1942 and 1945.

Before the discovery of antimalarial drugs, malaria was a common affiction in ancient times and was a constant obstacle to progress and development. It caused a great loss of human lives in Africa, Asia, the United States and also parts of Europe. Some scholars firmly believe that malaria was one of the factors which contributed to the fall of Roman empire. It is even said that the ravages of malaria and the lack of adequate supplies of quinine played a decisive role inthe collapse of the German armies during World War 1.

The discovery of antimalarial drugs opened a new chapter in world history. The bulk of the commercial supply of the drug is derived from the bark of several species of Cinchona, a native to the Andean highlands of South America, extending from Bolivia to Colombia. The important species yielding the drug are: calisaya or yellow bark of commerce (C.calisaya Wedd.); ledger bark or ledger hybrid bark. C.ledgeriana Moens (= C. calisaya Wedd. var. ledgeriana Howard); loxa or pale bark ofcommerce (C.officinalis L.) and red bark of commerce (*C.succirubra* Pay.). In addition to these, *C.micrantha* Ruiz. & Pav.and *C.nitida* Ruiz & Pav. of of Peru and Ecuador and *C.pitayensis* of Colombia also contain the alkaloid, *C. ledgeriana* gives the highest yield of quinine, producing up to 16 per cent in the bark.

Cinchona spp. are evergreen shrubs or trees with opposite, simple entire leaves and interpetiolar stipules. Small fragrant flowers are borne on terminal panicles. The capsular fruits are oblong to ovoid-lanceolate (Figure 15.2).

The plants are found growing at altitudes ranging from 760 to 2750 m (seldom below 300 m), preferring cool mountain slopes with an abundant and well distributed rainfall of over 220 cm. The plants grow best on light, well-drained, virgin forest soils rich in organic matter, with a pH of 4.5 - 6.5 . Propagation is either by seeds or by vegetative means, i.e. grafting and cuttings.

At present, *Cinchona* is cultivated on a large scale in India and Indonesia. A limited supply is also obtained from Tanzania, Sri Lanka and Burma (Figure 15.3).

The bark of *Cinchona* roots contain the highest concentration of total alkaloids, but the bark of the trunk is the richest source of quinine. The harvesting of the bark may be undertaken from the fourth year onward but the alkaloidal content of the bark reaches its maximum only after 10-12 years of planning. Bark is removed by uprooting the trees when 12 years of age or by cutting the stem above ground and then stripping the main stem and branches. The packed bark is subsequently shipped to the drug manufacturers.

Nearly 30 alkaloids have been isolated from Cinchona-spp. of which the most important are; quinine ($C_{20}H_{24}O_2N_2$), quinidine (isomer of quinine), cinchonine ($C_{19}H_{22}N_2O$) and its isomer cinchonidine - all four collectively known as 'totaquine'. The various alkaloids exist in the bark in combination with cinchotannic acid, quinic acid, free organic acids, tannins, colouring matters, gums, starch, vegetable matter and traces of volatile oils. Other less important alkaloids are; cinchotine, javanine, hydroquinine, hydroquinidine, cusconine, cusconidine, cuscamine and cuscamidine.

In addition to its use for the treatment of malaria fevers, it is also valuable as a tonic and an antiseptic. Quinine, quinidine and their compounds are employed in insecticides for the preservation of fur, feathers, wool, felt and textiles. The residual bark of Cinchona, left after the extraction of alkaloids, is used as a tanning material. The alkaloid quinidine is a cardiac depresant.

Several species of Remijia (family Rubiaceae), particularly R.purdieana Wedd. and R.pedunculata Flueck., are promising substitutes for Cinchona and contain substantial amounts of quinine and quinidine in the bark. Besides these, roots, stem and leaves of Dichroa febrifuga Lour. (Saxifragaceae) and leaves of Chamaebatia foliolosa Benth. (Rosaceae) are also sources of these alkaloids.

Cinchona has lost much of its importance through the widespread use of synthetic antimalarials free from some of the disadvantages of natural quinine. Plasmochin, quinachrine hydrochloride (atabrine), plasmoquine and chloroquine are prominent among the large number of compounds having anti-malarial properties.

Digitalis sp. Linn. (x = 7)   Foxglove
Family: Scrophulariaceae

Digitalis was used by the early herbalists for the treatment of epilepsy, wounds and other ailments. By using a leafy material obtained from the Witch Woman of Shropshire', William Withering succeeded in curing dropsy (unwanted accumulation of liquid in the body cavities)- now known to be a manifestation of heart disease. He published the first scientific account of foxglove and some of its medicinal virtues in 1785. But its true therapeutic value was recognised only in the earlier part of the twentieth century and it is now regarded as a pwerful medical agent controlling the myocardial tissue of the heart.

Thedrug is obtained from the dried leaves of the common foxglove, D.purpurea L., and wooly foxgloe, D. lanata Ehrh., native of central and southern Europe. The plants are now cultivated on a commercial scale in the United States, Central Europe, England and Argentina. In India, D.purpurea is cultivated chiefly in Kashmir and the Nilgiri hills, while D.lanata is grown in Kashmir at altitudes of above 2100 m and also at Chakrata (Uttar Pradesh).

The plants are beautiful, biennial herbs with rosette-like leaves and are easily recognised by their pendant, zygomorphi, tubular; purple or yellow flowers each conspicuously spotted on the inner bottom surface of the tube (Figure 15.4). In D.lanata, the flowers are smaller, hairy and cream, yellow or purple coloured.

Leaves of D.purpurea are the largest and most commonly used. They are picked by hand. Collection made during the first year contain the highest percentage of glycosides. Harvesting should be accomplished before flowering and the leaves must be quickly and thoroughly dried at temperatures not exceeding 60°C. Sun dried leaves retain their activity for a longer period. Dried leaves after carefull packing are shipped for export.

The active constituents of digitalis are mainly confined to the epidermal and subepidermal collenchyma and the endodermal cells of the vascular bundles. The total concentration of different glycosides in the leaves is about one percent. It contains a number of chemically and physiologically related cardiac glycosides. The leaves also contain saponins, tannis, gallic,formic, acetic, lactic, succinic and benzoic acids and about 1.2 per cent of various saturated and unsaturated fatty acids.

The physiologically most active glycosides of the leaves of D.purpurea, namely digitoxin, gitoxin and gitalin are known to be derived from the naturally occuring purpurea glycoside A, purpurea glycoside B and purpurea glycoside C* respectively by the loss of a glucose residue. Digitoxin, gitoxin and gitalin, on further hydrolysis, give rise to the non-sugar portion (aglycones or genins) dgitoxigenin, gitoxigenin and gitaligenin respectively. It has been reported that the aglycones are weaker in cardiac action than the glycoside from which they are derived. Digitoxin is the most potent of the digitalis glycosides, its activity being 1000 times more than that of powdered digitalis. Digitalin is another active cardiac glycoside obtained from the seeds of D.purpurea.

D.lanata has stronger medicinal properties and its side effects are not as toxic as D.purpurea. The ac tive glycosides of the leaves are digitoxin, gitoxin and digoxin, being derived from the naturally occurring or primary glycosides, lanatoside A, lanatoside B and lanatoside C respectively (also known as digilands A, B and C). Lanatoside C has no counterpart in D. purpurea. On further hydrolysis, digitoxin, gitoxin and digoxin yield digitoxigenin, gitoxigenin and digoxigenin. Digoxin produces the same cardiac effect as digitalis, being 300 times more potent than that prepared from digitalis leaves.

Because of its stimulatory action on the heart, digitalis is often employed in the treatment of circulatory disorders. As it regulates the tone and rhythm of the heart beat, the contraction is made strong and regular. As a result more blood is sent out from the heart, thus helping in circulation . It is used as a myocardial stimulant in congested heart failure. It improves the blood supply to the kidnesy and, therefore, acts as a diuretic and removes renal obstruction.

Poisoning in human beings results from overdoses of the drug. Symptoms include nausea, diarrhoea, abdominal pain, gross disturbances in heart beat and pulse, various mental irregularities, drowsiness or or tremors and even convulsions.

Ephedra spp. (x = 7)   Ephedrine
Family:Ephedraceae (Gymnospermae)

The therapeutical action of Ephedra was known to the Chines more than 5000 years ago under the same 'Ma Huang', 'yellow hemp'. Even in the Vedic period some of its species are believed to have been used as divine plants. Although the alkaloids were isolated in 1885 by Yamanashi (ephedrine in pure form was isolated by N.Nagai in 1887), their pharmaceutical value remained obscure until 1923. It was through the work of Drs. K.K.Chen and C.F.Schmidt that the role of ephedrine as a sympathomimetic was discovered. The alkaloid ephedrine physiologically and chemically resembles epinephrine (adrenalin), a hormone-like substance having a stimulatory action on the sympathetic nervous system. Since the classical research of Chen and his associate, five species of Ephedra, namely E. sinica Stapf, E.equisetina Bunge, E. gerardiana Wall., E.major Host, and E.distachya L. have been found to be the chief sources of the drug Ephedra is grown on a large scale in China, India, Spain, U.S.A., Kenya and Australia.

The leafless plants have a shrubby appearance and resemble the common horsetails, occurring abundantly in arid regions (Figure 15.5).

The potent alkaloid ephedrine is extracted from the dried or fresh branches of the stem. It has been estimated that the alkaloidal concentration increases with the age of the plant and reaches its peak when plants are nearly four years old, still in flowering. Among the Indian species, E.major is the richest source of ephedrine and contains over 2.5 per cent of total alkaloids.

The major alkaloid ephedrine ($C_{10} H_{15} ON$) occurs in combination with several other alkaloids, such as d-and-l-pseudo-ephedrine (isoephedrine) and ephedine. Ephedrine, however, constitutes nearly three-quarters of the total alkaloids.

Zephrol containing ephedrine hydrochloride has been employed in the form of nasal drops to relieve nasal and sinus congestion and also for the treatment of bronchial coughs. The use of ephedrine preparations in asthma, hay fever and colds are very popular. It has been also used to control night wetting (urinating shile sleeping in children). When administered intravenously, it stimulates thesympathetic nervous system and counteracts, overdosages of depressants, thus causing an immediate and pronounced elevation in blood pressure.

Overdosages of ephedrine, however, cause headache, sweating, insomnia, nausea and vomiting.

At present a long list of synthetic derivatives of ephedrine are sold on the drug market, e.g. benzedrine, dexedrine and pervitin. They have been used for alertness and wakefulness by pilots, motorists and others whose occupation requires long hours of physical exertion.

They also seem to excite and impart thrills to their users, resulting in habituation.

Papaver somniferum Linn. (n = 11)

Opium poppy, white poppy

Family: Papaveraceae

Opium and its derivatives-particularly morphine and heroin are the most dangerous of the habit-forming narcotic drugs and are most difficult to give up. Taken in moderate quantities under the advice of a physician, they are legitimate remedies and afford relief to millions of sufferers. Opiates remain unexcelled as hypnotics and sedatives, and are freauently used to relieve pain and anxiety; they cause mental and physical relaxation and often induce badly needed sleep. At the same time, they have become a curse to mankind being smoked, eaten, sniffed or injected by untold millions of unfortunate addicts of all religions and nationalities all over the world. Addiction to opium and its derivatives is so consuming that people become slaves to the habit and may commit crimes to support it. The immediate effects are pleasurable, inducing dreams and visions. However, continued use of opium and its derivatives leads to physical, mental and moral degradation, the addict in due course becoming a victim of delirium and death.

Opium eating was once quite common in Persia (now Iran), Asia Minor and India and opium smoking in China and India. There are still a great many opium eaters and smokers in China, Egypt and India, where opium is grown as a crop. Most drug addicts in the West today use heroin. Even today, opium dens are flourishing throughout the world, although opium consumption is illegal. In recent years opium addiction has greatly diminished owing to strict governmental control of the cultivation, distribution and export from the producing countries.

Crude commercial opium represents the air dried congealed or coagulated latex obtained from the unripe capsules of Papaver somniferum, believed to be a native of Asia Minor (Turkey) where it often grows as an escape. Opium was known to the ancient Greeks, Romans and Egyptians. It had reached Persia, India and China by the eighth century. In India, cultivation of poppy began during the early sixteenth century and was a profitable article of trade. The East India Company in 1767 started a flourishing business, exporting opium from India to China. The trade developed into such a great menace that the Chinese Government banned the import of the drug. The ban was one of the causes of the so-called 'opium war' of 1839-42 between Great Britain and China.

The opium poppy is also cultivated as an ornamental plant in many parts of the world. It is an erect, annual, glaucous herb, 30-100 cm tall. All plant parts contain latex. The leaves are ovate-oblong, with leaf bases embracing the stem, and are often shallowly pinnately lobed. The flowers are large and showy and are white or sometimes purple or scarlet in colour (Figure 15.6A). The fruit is a typical capsule developing from a multicarpellary ovary having parietal placentation.

The capsules are very large, ovoid or globular and topped by the persistent stigmatic disc with deep marginal lobes, dehiscing by means of valves or pores (Figure 15.6B). The seeds are small with a minute embryo in an oily endosperm.

In India, the opium poppy is cultivated as a rabi (winter) crop, the seeds being sown in October-November and the opium collected the following March-April. Opium poppy is grown in almost all types of soil but it prefers a well-drained sandy loam. The plant cannot tolerate extreme cold.

At present opium poppy plants are extensively cultivated in India. Turkey and U.S.S.R. A small amount is obtained from Yugoslavia, Bulgaria, Afghanistan, Pakistan and Japan. India is the only opium exporting nation. Opium poppy is cultivated in the states of Madhya Pradesh, Ut'ar Pradesh and Rajasthan.

To harvest the 'resin', incisions are carefully made into the maturing capsules from the bottom upwards with the help of specially designed tools. During lancing, deeper cuts are avoided so as to prevent the flow of milky latex among the seeds. The operation is carried out in the late hours of evening and before sunset. Exudate droplets collect on the surface of the capsule and are allowed to remain there overnight. The coagulated latex is scraped off before sunrise. Thick latex is stored in perforated metallic or earthen pots kept in a slightly tilted position to drain off water. Raw opium is then dried in the sun, which makes it viscid and dark brown, developing a characteristic odour. The thick mass is kneaded by hand into balls, cakes or opium bricks and wrapped in poppy or Rumex leaves for further slow drying.

Crude opium contains a large number of alkaloids, about 35 in all, besides other components such as gum, rubber, resin, oils, moisture, pigments, meconic acid, and other extraneous matter. Among the most important alkaloids found are: morphine ($C_{17}H_{19}O_3N$), codeine ($C_{18}H_{21}O_3N$), thebaine ($C_{19}H_{21}O_3N$), papaverine ($C_{20}H_{21}O_4N$), noscapine, formerly called marcotine ($C_{22}H_{23}O_7N$), and narceine ($C_{21}H_{28}O_8N$).

Morphine is physiologically the most active of all the alkaloids; it was isolated in 1806 by Serturner. It is the principal alkaloid constituting 8-13 per cent of opium by weight. Because of its sleep and dream inducing properties, the alkaloid was named after Morpheus, the god of dreams. Morphine is essentially an analgesic and sedative. It is well known as a pain reliever. Heroin (diacetylmorphine) is a more powerful analgesic than morphine. A large number of new drugs with morphine-like activity have now been synthesised, prominent among which are; pethidine, dionin, metapon and the methadone group. Morphine is also used in a number of cough medicines, for allaying diarrhoea and vomiting, and to reduce blood pressure and bleeding. Codeine resembles morphine qualitatively, but is slightly milder in action as an analgesic. It is used in whooping cough medicines as codeine sulphate and codeine phosphate.

The seeds of the opium poppy are, however, free from the narcotic constituent and are used in food. They are quite nutritious and have a pleasant nutty flavour and are often added to cakes or sprinkled on bread. The seeds are also a source of a fatty oil (poppy oil) which is used in India in the preparation of sweetmeats, curries, etc.

Rauvolfia serpentina Benth. ex Kurz. (n = 10, 11, 12, 22)
Rauwolfia
Family: Apocynaceae

The dried roots of Rauvolfia serpentina have long been used by the people of India as a cure for epilepsy, high blood pressure, insanity, intestinal disorders, cardiac diseases, snake-bite and as an anthelmintic. Some of the active alkaloidal constituents, e.g. ajmaline, ajmalinine, ajmalicine, serpentine and serpentinine were isolated for the first time by the Indian chemists Siddiqui and Siddiqui in 1931. Indian rauwolfias have now assumed a leading position in modern medicine, especially since the isolation of the main alkaloidal constituent, reserpine, by Muller, Schlittler and Bein of the CIBA Laboratories, Switzerland in 1952. Reserpine was the first tranquiliser to be used for the treatment of schizophrenia and other forms of mental disorders. Being a hypotensive agent, reserpine is widely employed today for hypertension (high blood pressure).

The drug rauwolfia is derived from the roots (especially the bark) of different species of Rauvolfia, named in honour of Leonhard Rauwolf, a German physician of the sixteenth century. Five species have been recorded in India, of which R.serpentina has attained a great reputation as a medicinal plant. R. tetraphylla L. and R.vomitoria Afzel. are two other species that are used for extracting reserpine in America and Africa respectively. The reserpine content of R.vomitoria is, however, twic that of R.serpentina.

R.serpentina is an upright , perennating, evergreen, glabrous undershrub with tuberous roots with a characteristic slightly wrinkled and coarse surface. The root bark is greyish yellow to brown and displays irregular longitudinal fissures. The leaves are simple, glabrous, lanceolate or abovate and are generally in whorls of three to four, crowding the upper part of the stem. The inflorescene is generally terminal but sometimes axillary, and usually consists of dense crowded cymes (Figure 15.7).

R.serpentina grows wild in India, Bangaladesh, Sri Lanka, Burma, Thailand, Indonesia and Malaysia. The plants is found in almost all parts of India from Kerala to the Himalayan foothills, except Rajasthan province. It is widely distributed in the sub-Himalayan tract (up to an elevation of about 1000 m) and in the lower ranges of the easern and western ghats and in the Andamans. Collection from wild sources has decreased considerably in recent years. Experimental cultivation has been undertaken in many parts of the country such as Uttar Pradesh, Maharashtra, Bihar, Jammu and Kashmir, Tamil Nadu, Kerala, Central India and Gujarat.

The plant grows in tropical or sub-tropical regions, benefiting from the monsoon rains. It may be grown almost anywhere at low or medium elevation where rainfall is not less than 75 cm. Rauwolfias flourish in hot humid conditions and can be grown both in the open and in partial shade. Soils with plenty of humus and a pH 4.0-6.3, are desirable for good growth. The plants are best raised from root-cuttings, but seeds and stem cuttings can also be used for propagation.

For commercial exploitation, roots are generally gathered two to three years after planting. It has been estimated that the alkaloidal content of the roots harvested after the shedding of leaves is far richer than the roots dug out in August. After harvesting, the roots are cleaned, air-dried and packed for shipment in airtight containers.

The total alkaloid content of the root varies from 1.7 to 3.0 percent, of which the bark alone accounts for nearly 90 per cent. The leaves and stems contain small amounts of alkaloids. A large number of alkaloids (80 or more) have been isolated from various species of Rauvolfia. Reserpine ($C_{33}H_{40}N_2O_9$) is pharmacologically the most potent. Other important alkaloids are; reserpinine, rescinnamine, deserpidine, deserpideine, serpentine, serpentinine, ajmaline, ajmalinine, ajmalicine, isoajmaline, rauwolfinine and yohimbine.

In addition to the uses of the drug mentioned earlier, it is known to stimulate uterine contraction and, therefore, is recommended for use in childbirth. An extract of the leaves has also been employed as a cure for the opacity of the cornea.

Strychnos nux-vomica L. (n = 12)   Nux vomica

Family: Loganiaceae

The dried ripe seeds of Strychnos form the chief botanical source of the valuable alkaloids that have acquired prominence more as a poison than for medication. The use of Strychnos as a virulent arrow poison (curare) has been known since antiquity. The medicinal and toxicological properties of the drug are mainly exhibited by the alkaloid strychnine.

Commercial supplies of the drug are obtained from S. nux-vomica, a native of southern Asia and Australia, and St. Ignatius's bean (S. ignatii Berg.) indigenous to the Philippine Islands. At present India, Sri Lanka, Malaysia, China and Australia are the chief producing countries. S. toxifera Schomb. ex Benth. and S. Castelnaei Wedd., are two well known sources of arrow poisons in South America.

The plants are woody vines or small trees which bear large berries resembling a mandarin or Chinese orange in shape and colour, each fruit containing from three to five greyish seeds (Figure 15.8). The seeds are hard, large circular or flattened structures having a bitter taste. The silky white sheen or lustre of the seeds is due to the presence of many closely appressed hairs (Figure 15.9).

Seeds are removed from the glutinous pulp either by washing or llowing the pulp to rot away. After a thorough washing and drying, the seeds are marketed. The seeds contain a high percentage of alkaloids (1.53-3.42 per cent) and are the principal source of the drug. Other plant parts such as old roots, wood, bark, leaves, blossom and the fruit pulp also contain varying amounts of alkaloids. The major alkaloids are strychnine and brucine. Besides these, the seeds also contain small amounts of strychnicine, vomicine, a-colubrine, B-colubrine and pseudostrychnine, which occur in combination with the glycoside loganin.

The drug is used for the treatment of nervous disorders and paralysis in minute dosages. Higher dosages are employed for destroying stray dogs and agricultural pests such as rats, rabbits, foxes, etc., the drug often being fed mixed with cereal flour. Strychnine and other alkaloids function as powerful stimulants on the central nervous system, especially of the spinal cord. Strychnine has been used as an antidote for barbiturate poisoning or other depressants. Since strychnine and brucine have a stimulating effect on the gastro-intestinal tract, the drug is often used as a tonic. Higher dosages of the drug produce muscular twitching or convulsions and, therefore, should be used with utmost caution.

Contd...P/27.

The fruit pulp is, however, non-poisonous and is eaten by birds, cattle and monkeys.

Claviceps purpurea (Fr.) Tul. Ergot
Ascomycetes (group Hypocreales)

The dreaded illness 'ergotism'* caused by the prolonged consumption of ergotised rye bread, stimulated extensive research on the biochemical nature of the fruiting body, the sclerotium. Although the cause of the disease was known in 1597, the important alkaloidal constituent was isolated from the crude drug only in 1906. Even today, blighted rye grains are eaten by peasants faced with hunger.

Commercial ergot represents the dried fruiting body, the sclerotium, parasiting only the ovary of the developing flowers of Secale cereale L. and other grasses. The sclerotium is a purple, dark brown or black, curved cylindrical structure consisting of a pseudoparenchymatous mycelial mass rich in oil lobules (Figure 15.10).

Ergot is a rich source of a number of pharmacologically active alkaloids - derivatives of either lysergic or isolysergic acids. Of the various alkaloids the best known physiologically active constituents are: ergotoxine (isolated by Berger and Carr in 1906), having an adrenolytic activity; ergotamine (A. Stoll in 1918), with a therapeutic use in obstetrics and ergonovine (Dudley and Moir in 1935), possessing the oxytocic principle. Today, all the naturally occurring alkaloids have been synthesised. In addition to the alkaloidal fraction, ergot contains sclererythrin (a red or violet pigment), ergosterol, clavicepsin, ergochrysin, ergoflavin, inorganic salts and large number of bases and amino acids.

The older term 'ergotoxine' found in the literature refers to an alkaloid complex consisting of three alkaloids (ergocryptine, ergocornine and ergocristine). Ergometerine and ergobasine are synonyms for ergonovine.

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*Ingestion of smaller dosages of ergot everyday over a period of several weeks to a few months produces gangrenous ergotism (necrosis of tissues of the extremities) and convulsive ergotism.

Contd...P/28.

Owing to its stimulatory action on the muscular coats of the stomach, the intestine, the uterus and the blood vessels, ergot has been frequently used in obstetrics for accelerating childbirth. It is used extensively to increase the blood pressure (as a hypertensive agent) and also for controlling uterine haemorrhage after childbirth. Heavy dosages of ergot cause nausea, vomiting, diarrhoea, and sometimes lead to unconsciousness and collapse. Lysergic acid diethylamide (LSD), however, is one of the worst habit-forming drugs known, and is suspected of causing chromosomal aberrations (although unequivocal evidence has not yet been produced).

Commercial supplies of ergot comes chiefly from central Europe, Spain, Portugal and from North American sources.

ANTIBIOTICS

In the twentieth century, tremendous progress has been made in the development of new disease preventing agents - the antibiotics. These chemical substances are produced and excreted by the living organisms in the course of their metabolism and are capable of killing other organisms. These 'miracle drugs' are chiefly obtained from microorganisms.

The lowly moulds were employed as healers to relieve festering ulcers by the Chinese thousands of years ago. Also, several centuries ago, North American Indians are known to have used both soil and rotting woods for the treatment of would infections and healing festered cuts.

Pasteur and Joubert reported in 1887 that the growth of anthrax bacteria was slowed down by contaminating organisms. Again in 1889, Emmerich and Low revealed that a substance isolated from Pseudomonas aeruginosia (Schroeter) Migula had a similar inhibitory action. But the clinical importance of their work remained unrecognised for quite some time. It was only in 1928 that Sir Alexander Fleming, a bacteriologist working at St. Mary's Hospital, London, incidentally found that the green mould contaminating a culture of Staphylococcus caused lysis (dissolution and destruction) of adjacent bacterial colonies which were known to cause serious diseases in man (Figure 15.11).

Contd...P/29.

After careful experimental studies, he discovered that the lysis was due to a chemical substance produced by the fungus (Figure 15.12). The name 'penicillin', after the green mould Penicillium, was given to the drug which won him fame and recognition for his scientific contribution. The discovery of penicillin was the forerunner of the modern antibiotic age.

Fleming's early work on penicillin was continued by Dr. Howard Fiorey, Ernst Boris Chain, N. G. Heatly, and others at Oxford. They demonstrated the remarkable power of penicillin to control various animal diseases caused by several pathogens such as Staphylococci, Streptococci and Pneumococci. In 1941, the drug penicillin was first administered to human beings with spectacular results. Since that time many antibiotics have been developed.

The original penicillin was simply the filtrate of the nutrient broth upon which Penicillium notatum West. was grown. With the collaboration of scientists from the Northern Regional Research Laboratory of the United States Department of Agriculture, Florey, in 1942, succeeded in perfecting techniques for the mass production of penicillin. Selected strains of P. chrysogenum Thom are grown in small culture flasks containing standardised corn-steep liquor and lactose (soyabeam-meal for most actinomycetes). These cultures are, in turn, used to inoculate large 'seed tanks' which can be further employed to inoculate huge vats containing thousands of gallons of nutrient media or both. Sterile air is continuously bubbled through the mechanically agitated culture medium. After maximum growth of the fungus, the nutrient broth is filtered, subjected to solvent extraction from which penicillin is precipitated out by suitable techniques. Crystallisation, purification, standardisation and packaging are done quickly under aseptic conditions. The purified penicillin was found to be a thousand times more potent than Fleming's original material and was far more stable. By 1945, penicillin become available to almost all hospitals throughout the world. One part of pure penicillin in fifty million parts of water is sufficient to kill most microbes, which makes penicillin the physician's most potent weapon for curing many infectious diseases.

Contd...P/30.

Penicillin has also been artificially synthesised. So far, none of the other antibiotics has equalled penicillin and it is still the most powerful and least toxic antibacterial agent. Today, there are several types (at least a dozen) of penicillins, derived from strains of P. notatum and P. chrysogenum. Penicillin usually comes from the factory as the potassium or sodium salts of penicillin G (benzylpenicillin).

Today, about 800 or more antibiotics have been described; of these the penicillins, streptomycin, chloromycetin (chloramphenicol) and the tetracyclines have been widely used in medicine. Bacitracin and polymyxin are two of the relatively few antibiotics obtained from bacteria. Antibiotics have not been effective against most virus diseases. Isolation of jawaharine from Aspergillus niger van Tieghem (Mitra, 1968) created a sensation among research workers because this antitumour antibiotic was reported to have antiviral activity.

Apart from several microorganisms, antibiotics have been isolated from some of the algae, lichens, a number of flowering plants and also from animal sources. More than half of the antibiotics discovered are produced by antinomycetes. The available space in the text does not permit a detailed discussion of individual antibiotics. Only a few of them are, therefore, listed, along with data about their discovery.

Antibiotics are used extensively in veterinary medicine. They are used also in livestock feed as a supplement, in the treatment of plant diseases, and in the preservation of biological material.

Contd...P/31.

given Antibi

Table 1. Important antibiotics obtained from plant sources

Name of the antibiotic	Year	Discovered by	Source
Penicillin	1928	Alexander Fleming	Penicillium notatum West. P. chrysogenum Thom
Streptomycin	1943	Selman A. Waksmath et al.	Streptomyces griseus (Krainsky) Waksman & Henrici
Bacitracin	1945	H. Anker B. A. Johnson F. L. Meleney	Bacillus subtilis Cohn amend.
Chloromycetin or Chloramphenicol	1947	P. R. Burkholder	Streptomyces uenezuelae Ehrlich, Gottlieb, Burkholder
Polymyxin	1947	Robert G. Benedict & A. F. Langlykke	Bacillus polymyxa (Prazmowski) Migula
Aureomycin	1948	B.M. Duggar et al.	Streptomyces aureofaciens Duggar
Neomycin	1948	Selman A. Waksman & H. A. Lechevalier	S. fradiae Waksman & Curtis
Hydroxystreptomycin	1950	Robert G. Benedict et al.	S. griseocarneus Benedict at al.
Terramycin	1950	A. C. Finley et al.	S. rimosus A. C. Finley et al.

Table 2. Some other important drug plants*

Name of the drug	Botanical name	Family	Place of origin	Part used	Active constituents	Uses
1	2	3	4	5	6	7
Aconite	Aconitum napellus L.	Ranunculaceae	Mountains of Europe and Western Asia	Dried roots	Aconitine, aconine, benzoylaconine	Externally used for neuralgia and rheumatism; internally to relieve pain and fever
Vasaka	Adhatoda vasica Nees.	Acanthaceae	India	Dried leaves	Vasicine, adhatodic acid, essential oil	Used as an expectorant, Component of glycodin vasaka - a cough mixture used in India
Wormseed	Artemisia maritima L.	Compositae	Pakistan	Dried immature leaves and flower heads	Santonin	Anthelmintic, more effective against round worm than threadworm; also used for dropsy
Senna	Cassia angustifolia Vahl (Indian senna)	Leguminosae	Somalia and Arabia	Dried leaves	Aloe-emodin, chrysophanic acid kampferin, sannoside A and B	Laxative used in habitual constipation
	C. acutifolia Del. (Alexandrian senna)	Leguminosae	Tropical Africa	Dried leaves		
Ipecac	Cephaelis ipecacuanha (Brot.) Rich.	Rubiaceae	Central and South America	Dried roots and rhizomes	Emetine, cephaeline	Used as expectorant and emetic; also in the treatment of amoebic dysentery and diarrhoea.

1	2	3	4	5	6	7
American-Wormseed	Chenopodium ambrosioides var. anthelminticum (L.) Gray.	Chenopodiaceae	West Indies, and Central & South America	Fruits	Ascaridol	Anthelmintic- for hookworm infections
Licorice or liquorice	Glycyrrhiza glatra L.	Leguminosae	Southern Europe and Middle East	Dried roots and rhizomes	Glycyrrhizin or glycyrrhizic acid (glycoside), glycyramarin, (bitter principle)	Used as an expectorant and demulcent: for flavouring candy and tobacco
Ginseng	Panax quinquefolia L.	Araliaceae	Northeastern U.S.A.	Dried roots	Panaquilon (glycoside)	Used as cure for almost all diseases in China
	P. ginseng C. A. Mey.	Araliaceae	Korea and Manchuria	Dried roots		
Psyllium	Plantago indica L.	Plantaginaceae	Southern Asia	Dried husk and entire ripe seed	Xylin (mucilaginous content)	As cathartics - for the treatment of chronic constipation.
	P. ovata Forsk.					
Podophyllum	Podophyllum emodi Wall.	Berberidaceae	India	Dried roots and rhizomes	Podophyllotoxin, podophylloresin, picropodophyllin, podophyllic-acid	Used as an evacuant or purgative in cases of chronic constipation; also being used for tumorous growths
	P. peltatum L.		Eastern U.S.A.			

1	2	3	4	5	6	7
Cascara	Rhamnus purshiana DC.	Rhamnaceae	North America	Dried tree	Emodin, iso-emodin, aloe-emodin, chrysophanic acid	Tonic and Laxative
Rhubarb	Rheum officinale Baill.	Polygonaceae	China	Dried rhizome and root:	Emodin, iso-emodin, aloe-emodin, chrysophanic acid	Tonic and laxative
	R. palmatum L.					
Strophanthus	Strophanthus hispidus DC.	Apocynaceae	Tropical Africa	Seeds	Strophanthin-G (ouabain). strophanthin-K.	Cardiac or heart stimulant
	S. kombe Oliv.					
Chaulmoogra oil	Taraktogenos kurzii King (=Hydnocarpus kurzii (King) Wrlg.)	Flacourtiaceae	Burma and South eastern Asia	Seeds	Chaulmoogric acid, hydnocarpic acid, goric acid	Treatment of leprosy
Ashvagandha	Withania-somnifera Dural.	Solanaceae	India	Dried roots	Nicotine, somniferin, somniferinine, withananine	Diuretic; used in rheumatism and applied to ulcer carbuncles, and painful swellings.

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* Betel, cocaine, hashish, ololiuqui and peyote are included in the chapter on fumitory and masticatory materials.

BUFFER SOLUTIONS

Dr.S.K.Mohapatra
Reader in Chemistry

What are buffer solutions?

Analytical processes involving separation, precipitation etc. required not only the knowledge of pH of the reaction medium but also its rigid maintenance during the course of changes. Many biological processes occur at specified pH. Enzymes have been found to be most active at a given pH value, known as the optimum pH for the particular reaction. (For most enzymes it is between 6 and 8). This requires the body fluids both extracellular and untracellular to be maintained rigidly within such pH limits. Experiments show that extracellular blood has pH 7.4 and intracellular blood has pH slightly lower than 7.0 These values are rigidly maintained inspite of addition of large amounts of acid in the form of hydrochloric, phosphoric, sulphuric and lactic acid during the normal process of metabolic activities. Carbon dioxide generated during oxidation of carbohydrates, fats and proteins dissolves in the bllod and forms carboric acid. How come then the blood is maintained at specific values? Co_2 is expelled through the lungs, and a part of other acids is excreted by the kidney as wine, while another part is neutralised by ammonia synthesized in the kidneys. But large fraction of the added acids or alkali (vegetation diets, fruits and drugs taken to gastric problems) is neutralised by what are known as buffer systems built in by nature.

Experiments show that such systems consist of a ~~mx~~ mixture an weak acid or base and one of its salts, and they have the ability to resist change in pH when small amounts of acids and alkalis are added.

Mechanism of buffer action: The mechanism of buffer action can be explained by taking a buffer system as acetate buffer which is a mixture of acetic acid and sodium acetate. When an acid is added, the salt reacts with it to form acetic acid.

$$CH_3COONa + HCl \longrightarrow CH_3COOH + NaCl$$

and when an alkali is added, it reacts with the acid to form the salt.

$$CH_3COOH + NaOH \longrightarrow CH_3COONa + H_2O$$

Thus the added acid is removed (neutralised) by the salt and the acid respectively. The acid formed does not dissociate appreciably in presence of the salt (common ion effect). Therefore the hydrogen ion concentration does not change or pH remains constant.

Physiologically important buffers:

Two-thirds of the buffer activity in vertebrates is carried out by phosphate buffer present in the kidney which is a mixture of KH_2PO_4 and Na_2HPO_4. KH_2PO_4 functions as the weak acid ($K_a = 6.2 \times 10^{-8}$) and Na_2HPO_4 functions as the salt and the reactions are

$$H_2PO_4^- + OH^- = HPO_4^{=} + H_2O$$

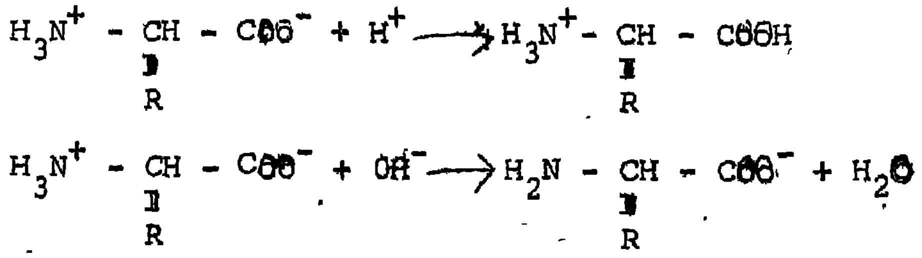
$$HPO_4^{=} + H^+ = H_2PO_4^-$$

The other third of the buffer activity is carried out by bicarbonate buffer which is a mixture of H_2CO_3 and HCO_3^- behaving as acid and salt respectively.

$$HCO_3^- + H^+ \longrightarrow H_2CO_3 \rightleftharpoons H_2O + CO_2 \quad \text{(expelled by lungs)}$$

$$H_2CO_3 + OH^- \longrightarrow HCO_3^- + H_2O$$

In the tissues, the buffer activity is largely carried out by the plasma proteins. The amenoacids constituting the proteins can neutralize acids and alkalis as follows.



$$H_3N^+ - \underset{\substack{|\\R}}{CH} - COO^- + H^+ \longrightarrow H_3N^+ - \underset{\substack{|\\R}}{CH} - COOH$$

$$H_3N^+ - \underset{\substack{|\\R}}{CH} - COO^- + OH^- \longrightarrow H_2N - \underset{\substack{|\\R}}{CH} - COO^- + H_2O$$

Besides both deoxygenated and oxygenated haemoglobin which are polyprotic acids (having at least five acid groups) can also and do neutralise added acids and alkalis.

$$H\,Hb + OH^- \longrightarrow Hb^- + H_2O$$

$$Hb + H^+ \longrightarrow HHb$$

(Haemoglobin ion)

Buffer range:

Each buffer solution functions effectively within a small pH range called its range. The pH of a buffer solution is given by the Henderson equation.

$$pH = pK_a + \log \frac{[Salt]}{[Acid]} \text{ where}$$

$pK_a = -\log K_a$ (K_a being the dissociation constant of the acid). Therefore pH of a buffer solution depends upon (1) the dissociation constant of the acid and (2) ratio of the concentration of salt and acid. When the ratio is 10:1 $pH = pK_a + \log 10 = pK_a + a$ and when the ratio is 1:10, $pH = pK_a - 1$. When the ratio is greater than 10:1 or less than 1:10 the effectiveness of the buffer declines. Therefore the effective range is $pK_a' \pm 1$. Thus acetate buffer has an effective range from 3.42 to 5.29 pK_a of acetic acid being 4.76 ; the phosphate buffer has a range 6 to 8 pK_a of KH_2PO_4 being 7.21.

Preparation of Buffer Solutions:

A buffer solution of a given strength and pH can be prepared as follows. Let us say we wish to prepare 0.2 M buffer of pH 5.4. First we have to choose an acid whose pK_a is around 5.4 . We refer to the dissociation constants of the acids. In this case we can either choose acetic acid (pK_a = 4 .76) or better monosodium - citrate (pK_a = 4.76). Next we have to find out the ratio of the concentration of the acid to the salt by using Henderson's equation

$$pH = pK_a + \log \frac{[Salt]}{[Acid]}$$

$$5.4 = 4.76 + \log \frac{[Salt]}{[Acid]}$$

Contd...4

$$\text{Or } 0.64 = \log\frac{[\text{Salt}]}{[\text{Acid}]} = \log\frac{[\text{disodium citrate}]}{[\text{monosodium citrate}]}$$

$$\text{or } \frac{[\text{Salt}]}{[\text{Acid}]} = \text{antilog of } 0.64 = 4.365$$

Now the buffer to be prepared should be 0.2 M which means the total concentrated, i.e. Salt + Acid must be 0.2 M. Therefore if the concentration of Salt = x M, the concentration of the acid has to be (0.2 - X) M.

$$\text{Hence } \frac{x}{0.2 - x} = 4.365$$

$$\text{Or } \quad x = 0.8730 - 4.365\,x \quad \text{or } x(1 + 4.365) = 0.8730$$

$$\text{Or } \quad x = \frac{0.8730}{5.365} = 0.1627 \text{ M}$$

$$\text{Or } [\text{Salt}] = 0.1627 \text{ M}$$

$$\text{and } [\text{Acid}] = 0.2 - 0.1627 = 0.0373 \text{ M}$$

Therefore the amount of salt to be taken is 0.1627 X 237 g (mol.wt. of disodium citrate) and amount of acid to be taken is 0.0373 X 214 g (mol wt. of monosodium citrate) and the volume made upto 1 litre.

Preparation of Buffer solutions of varying pH

Requirements:

1. Measuring flasks (500 ml) - 3
2. Measuring cylinders (400 ml, 50 ml. 25 ml) - 1 each
3. Reagent bottles - 9 nos.
4. Weighing bottle or glazed paper
5. Burette - (50 ml) - 1
6. Conical flasks (250 ml) - 6 nos.
7. Burette stand.
8. pH meter or Indicator soln.
9. Monosodium citrate
10. Disodium citrate
11. Sodium hydroxide
12. Chemical balance with weight box

Contd....5

Procedure:

(a) Prepare 500 ml each of 0.1 M solutions of monosodium and di-sodium citrate. Then prepare a series of buffer solution as follows.

Vol.of monosodium citrate soln.	Vol. of disodium citrate soln.
90 ml	10 ml
80 ml	20 ml
70 ml	30 ml
60 ml	40 ml
50 ml	50 ml
40 ml	60 ml
30 ml	70 ml
20 ml	80 ml
90 ml	10 ml

Measure their pH by any of the methods described in the previous unit and note in right column.

(b) Alternatively, take 25 ml of the monosodium citrate and neutralise to 10%, 20%, 30% ....to 90% by a standard alkali.

Preparation of some aqueous buffer solutions

(1) Acetate Buffer

(a) Acetic acid (0.2 M)

Dilute 28.5 ml of glacial acetic acid to 1 litre. This gives an approximately 0.5 M solution. Standardise this acid with 0.5 M NaOH solution and determine the exact strength. Then prepare an exactly 0.2 M solution of acetic acid by using the formula $V_1 X S_1 = V_2 S_2$

(b) Sodium acetate (0.2 M)

Dissolve 16.4 g of sodium acetate in 1 l of distilled water.

Contd...6

Vol. of acetic acid	Vol. of sodium acetate soln.	pH
94	6	3.5
82	18	4.0
56	44	4.5
30	70	5.0
20	80	5.2
12	88	5.5
10	90	5.6

(2) Phosphate Buffer

(a) 0.025 M KH_2PO_4
Dissolve 3.4 g of KH_2PO_4 in 1l distilled wat

(b) 0.025 M Na_2HPO_4
Dissolve 4.0 g of $Na_2HPO_4 \cdot H_2O$ in 1 ltr. distilled water.

Equal volumes of (a) and (b) gives a buffer solution of pH 6.85.

Variation of pH in the range 5.85 to 7.8 can be made by varying amounts of the two solutions as in the case of acetate buffer pH can either be measured or calculated from Henderson's equation.

Note: The number of molecules of water of crystallization on the sample and take them into account in calculating molecular weight.

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INSTRUCTION IN PLANT PATHOLOGY

M.P. Sinha

A century ago a German doctor, Rober, Koch, became fascinated by millions of rod-shaped bacteria in his microscope field. Could they be the cause of the dreaded anthrax that was killing hundreds of cattle and sheep throughout Europe. He dipped sharpened wooden splints in to some of the fluid from diseased animals and carefully insected the splints into his laboratory mice. In the morning the mico were all dead, and in their blood was the same kind of rod-shaped bacteria, providing evidence that these bacteria could indeed cause disease resembling anthrax. The procedures which Dr. Koch followed in providing evidence that bacteria associated with animals dead with anthrax were actually the cause of that disease have become known as Koch's postulates or Rules of Proof of pathogenicity. By these procedures many diseases of human and animals have been shown to be caused by specific microorganisms. Likewise, they have been utilized routinely by plant pathologists to prove that bacteria and fungi cause disease in plants.

A plant becomes diseased where an interaction between the plant and disease causing organism(micro-or-ganism)results in the impairment of the normal physiological functions of the plant. The severity of the disease or the extent of the interaction is a function of the influence of environment on the development of the plant, on the activities of the microorganism and on the interaction between the plant and the microorganism. Disease in plants, therefore, embodies many biological phenomenc. The following steps may be organised in order to study plant diseases.

1. Collection of materials (diseased plants)

A walk around the vegetable or flower garden, through the fields on in the woods during the growing season will reveal a variety of spots and blights on plant leaves and stems (symptoms). Predominants among these maladies are mildews, the rusts, and the smuts, which are easily recognized by the vast numbers of spores produced on the plant surface.

Make field trips in the late summer or late winter and observe plant foliage for mildews, rusts and smuts.Examine lesio in side with a hand lense.for the presence .of hyaline chains of spores of the powdery mildews,for dusty orange or redish brown " spores of the rusts or for brawnish-black masses of spores dust of the smuts.The whitish powdery mildews may be found in abundance on lilac,cucumber,grasses or legumes,Rusts abound on wheat,Oats,and grasses, an hollyhocks, and on some wild flowers. Smuts are less readily recognised by the casual observer but the common smut of corn may be found in nearly every field.

When you return to laboratory examine the specimens, microscopically,by cutting hand sections, staining in acid fuchin/ cotton blue for two minutes and mounting in glycerine. Press some leaf specimens and dry some smut galls for future use.

Expected Results:-The number of spores produced by these fungi is unbelievable. The powdery mildews derive their name from the"powdery" masses of spores piled on the leaf surface, In among these masses may be found the sexual fruiting bodies of this fungus,the perithe filled with ascospores. These black structures have beautiful appendages,easily seen with the stereoscopic microscope, The rusts have a variety of spores types, all produced by the hundreds in specialized fruiting structures. The black "dust" of the smuts, like-wise consists of hundred of tiny spores.

2. Isolation of plant pathogens(Bacteria and fungi)

Microorganisms suspected of causing disease in plants must first be isolated before pathogenecity can be demonstrated. The techniques used to isolate organisms vary with nature of the plant tissue invaded and the kind of microorganisms revolved. Many fungi and bacteria that cause disease in plants, may be isolated and grown niculture/pureculture in the class room

Procedure: a) Storage rot: Wash a fruit or vegetable,e.g apple or carrot,that is partially rotted. Immerse it in a dilute dis infestant(70% alcohol)for five minutes. Damp dry it with paper toweling and cut it open to expose the area between some rotted and healthy tissue. Transfer bit of tissue from this margin to PDA in petridishes,and space 4-5 pieces per plate. If examination for fungi only is desired,add two drops of 50% lactic acid to the plate before passing the agar. More precise methods for recovery of bacteria may be use,e.g. the serial dilution technique.Incubate the dishes at room temperature.Start observation after 48 hrs.

b) Leaf and stem lesions: Collect and wash spotted leaves or stem and immerse 1.2mm squares of tissue from the margin of of a diseased area in disnifestant.It may be necessary to reduce the time of immersion or strength of disnifestant for this tissue to avoid killing the causal organisms.Transfer bits of tissue to P.D.A. and incubate at room temperature.

Observation:- Start observation after 48 hrs. fungi for bacteria present in diseased tissue will grow on to the agar surface.Bacteria will be inhibited on acidified PDA. If a particular colony type is recovered consistently from diseased tissue of a certain plant it is circumstantial evidence that the organism may have caused the disease.

3. Preparation of PDA(Potato dextrose agar) media

Requirements

(a) Distilled or tap water	1000 ml.
(b) Sliced unpeeled potato	200 gm.
(c) Agar	20 gm.
(d) Dextrose	20 gm.

Procedure.Cook potatoes in 500 ml of water for 20-30 minutes. This should be half boiled.Filter the broth through clean cheese cloth or ordinary cloth to remove the potato debris. Dissolve the agar in another 200ml of water. If necessary warm the water slightly. Dissolve the dextrose in another 200ml of water. Mix the hot potato filtered broth,the agar solution and the dextrose solution in a 1000ml flask. Mix up throughly and add water to make 1000 ml. Plug the flask with non-abxrbant cotton and sterilize it in a pressure cooker for 30 minutes.

4. Stains:-

a) Acid fuchsion:- 0.5-1.00 gm of acidfuchsin crystals to be dissolve in water. If necessary may be warmed.

b) Cotton blue in lac to phenol.

Lactophenol solution is prepared and consists of 20 gms phenol (warmed until melted), 20 ml lactic acid, 20 ml glycerin, 20ml distilled water. To one-half of the lactophenol solution is added enough 0.5% cotton blue solution in distilled water to give a dark colour.

CONCEPT AND CONCEPT TEACHING AND LEARNING
AN OVERVIEW

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What is a Concept:

A concept is a class of stimuli which have common characteristics. These stimuli are objects, events or persons. A concept is ordinarily designated by its name such as pencils, bottles, pupil or freedom fighters, committed workers, and nasty places etc. All these concepts refer to classes or categories of stimuli. But some stimuli do not refer to concepts i.e. Subhash Bose, Sarala Das (Adi Kabi), Tagore's Gitanjali, Indo-Pak war of 1971, Annual Book Exhibition. These are particular stimuli (not classes of), persons or events A concept is not a particular stimulus but a class of stimuli, the difference is between all freedom fighters and Subhash Bose. The concept freedom fighters includes Subhash Bose, but it includes many other fighters as well. The concept freedom fighters excludes all other war fighters. It is to be remembered that concept does not refer to particular stimuli but to classes of stimuli. The concept is a very broad one and it can include fighters of different types who faught for attaining freedom from British rule in various ways. Similarly, the concept bottles of varied sizes, colouration and shapes, pencils, of different lengths, qualities and types.

Hence concepts are not always congruent with our personal experience, but they represent human attempts to classify our experience at least crudely.

Contd........2/-

Concept attributes:

An attributes is a distinctive feature of a concept and thus varies from concept to concept. For example: Red triangles which has two attributes: colour and form or shape. Colour can vary from concept to concept and, therefore, qualifies as an attribute. We can indeed have red squares, red rectangles, red trapiziums red parallelograms. A concept is lake. The chief attribute which distinguishes a lake from an ocean and sea, on one hand and from a pool and pond, on the other hand, in size. Size is one of its major attributes. Size qualifies as an attribute because it can vary from concept to concept. Of course there are other attributes of lake.

Attribute Values - Values are the particular variations an attribute may undergo. Colour is an attribute. It may have several values: red, white, blue, violet, black. Similarly form may have several values; rectangles squares, rohmbus, quadrangles. Concept vary in the number of values their attributes have. Some concepts have attributes with only two values. A student (a concept) can be a boy or girl, dead or alive, married or single. Other concept may have attributes with a range of value colour of an orange can vary from red-orange to yellow-orange. The colour, however, must not vary so much that we confuse an orange with a lemon or Mousumbi or shaddock. When an attribute has a wide range of values, the other attributes can be used to identify the concept in question. In identifying an orange the attributes of shape, size and texture can also be used.

Contd 3

Number of attributes:

The number of attributes varies from concept to concept. Red triangle has only two attributes-colour and form. Small red triangle has three attributes-size, colour and form. An orange, has four attributes-colour, size, form and texture. Some complex concepts have a dozen or more attributes such as socialism, human rights, democracy etc. As the number of attributes increases the difficulty of learning of concept increases. Scanning the values of a dozen attributes is strenuous and time consuming. Bruner and his associates suggest that to have easy learning the number of attributes can be reduced by attending to some attributes and ignoring others or by combining a number of attributes into a smaller number of patterns.

Dominance of the Attributes:

Among the attributes physical location is more dominant than the attributes of colour and form. Also colour form concepts such as red triangles are more dominant than number-colour concepts such as one red. Thus, dominance refers to the concept as well as to its attributes. Dominant concept has dominant attributes. Learning concepts with dominant attributes with fewer examples is earlier than learning concepts with obscure attributes.

Informally, it is observed that students usually attend to certain points in their description of a concept but ignore other points that are equally important. In placing the concept of starrs children may attend to the attribute of placement/visibility of

Contd 4

celestial bright bodies in the night sky and ignore the condition of twinkling, movement, size, colour etc. Teachers must give aural or visual emphasis to attributes which are obscure and yet important in identifying the concept. In defining concepts teachers traditionally resort to vocal inflection, hand and arm gesticulation, under-scorings, diagramming, drawing and so on, to make obscure attributes obvious or dominant. Unless this emphasis is provided,the student will learn some attributes and not others and, thereby, fail to learn the complete concept.

Types of Concepts:

Attributes combine in three different ways to produce three types of concepts: Conjunctive concepts, disjunctive concepts and relational concepts.

a) Conjuctive Concepts: The appropriate values of several attributes are jointly present. Ex-Three white half-shirts. It has three attributes (number,colour,form, joined together and each attribute has a particular value (respectively three, white, half-shirts). Conjuctive concepts are often the easiest to learn and to teach because of the additive quality of their attributes and values. Attributes and values are added together to produce a conjuctive concept. The student simply learn a list of attributes and appropriate values.

b) Disjunctive Concepts: It is the one that can be defined in a number of different ways. Attributes and values are substituted for one another.

Ex - a) Two figures and/or two circles.

b) Strike

c) Extra point in foot ball

Contd.....5

The attributes are form and number and the value of the number remains the same. The concept is disjunctive because the value of the form can change - it can be a circle or any form.

Disjunctive concepts are often difficult to learn because of the seemingly arbitrary equivalence of their attributes. Disjunctive concepts are, in effect, rules which the student must learn to apply to equivalent stimulus situations. But the situations are not equal or equivalent until given the level. Teachers must invest greater **effort** in the teaching of disjunctive concepts.

Relational Concepts: It is the one that has specifiable relationship between attributes.

Ex- Distance and direction are relational concepts. Distance specifies the relationship between two points; it refers to the separation of these points. Direction also specifies a relationship between two or more points; it refers to the movement from one to another point.

More examples - Time, many, few, average, longitude, mass, weight, mother, father etc. Relational concepts are more difficult to learn as the concept does not adhere in the attributes themselves. but in the particular relationships of the attributes. This sometimes creates lots of confusion in learning. For example, both the concept distance and the concept direction have as their attributes points in space and time. What distinguishes them is the difference in the relationship of the same attributes.

Contd.....6

What is Principle:

A principle is a statement of the relationship between two or more concepts. Principles are sometimes called rules or generalisations.

Ex- a) Rivers flow from hills to oceans.
b) Thirteen minus four equals nine.
c) The density of water is more than oil.
d) Three dimentional objects have six sides

The following statements are not principles:

a) Shyam likes Rahim
b) Rajiv claims he is stronger than any body in India.
c) Congress won the last election.
d) Who is afraid of Nandan Kanan Tigers.

In the above set of statements concepts are there but those do not have relationships; basing on those concepts no rules or generalization can be made only the proper arrangement of the concepts results in satisfactory learning of principles.

When to teach concepts:

The teaching and learning of concepts must be related to the students' level of intellectual development. In teaching concepts during the period of concrete operations (age 7 to 11), the teacher must remember that the learner's thinking is oriented towards concrete objects in the immediate environment, that the child relinquishes the physical attributes of objects one by one, and that each grouping (or schema)remains an isolated organisation. In the period of formal operations,the adolescent child is capable of hypotheticodeductive and propositional thinking.

Contd.....7

Thinking. Although the teaching of concepts can and does occur during both periods, the teaching of principles proceeds more easily during the later period. Because the child's school learning of concepts is limited by his preschool learning, the school must often provide corrective experience to exclude irrelevant and include relevant attributes. Teacher should be in a position to decide on which concepts students should learn first and which they should learn later.

Educational uses of concepts and principles:

1) Concepts reduce the complexity of the environment.
2) Concepts help us to identify the objects of the world around us.
3) Concepts and principles reduce the necessity of constant learning.
4) Concepts and principles provide direction for instrumental activity.
5) Concepts and principles make instruction possible.
6) Concepts can be sterectypes. The teacher must

Sometimes provide corrective experience for an additional use of concepts: Sterectypes. As concepts, sterectypes can sometimes be changed when the student is provided with a wider array of positive and negative examples then those which he has previously experienced.

The Teaching of Concepts

The teaching of concepts conform to the components of the basic teaching model. The process completes through seven steps steps 1 and 2 pertains to

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instructional objectives. Step 1 requires a statement of the objective, step 2, a type of task analysis. Step 3 provides the student with the appropriate entering behaviour. Step 4 through 6 are specific instructional procedures for concept teaching and step 7 deals with performance assessment.

Step 1 - Describe the performance expected of the student after he has learned the concept.

The expected performance is the correct identification of new examples of the concept. For the concept 'Satellites; the expected performance could be that when new examples of satellites given the learner will correct identify them. The description of terminal behaviour requires a performance quite different from rattling of definition. The point is that the description of the expected behaviour should not include the requirement that the student give a definition of the concept.

Describing terminal behaviour has two purposes. First the teacher has a means for assessing the adequacy of the performance and for determining the need for further instruction. The students' expected performance clearly indicates to the teacher and to the students the degree of adequacy the students are to attain a particular time. Second, the students have a way of assessing their own performance and of determining when learning is complete. The students' self-assessments then become a way of generating their own reinforcement.

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Step.2 Reduce the number of attributes to be learned in complex concept and make important attributes dominant.

In this step the values,number, dominance and relationship of attributes can be put to pedagogical use. The analysis of the concept is decided to teach.The determination of the values and number of attributes can be made before instruction is underway.The determination of dominance of the attributes requires experience and observation of important attributes students are likely to ignore. Then procedures for teaching the concept are to be devised in two ways. Some of the attributes can be ignored and focus must be on those which the teacher thinks most important and/or the attributes can be coded into fewer patterns. But for a complete understanding of the concept,the learner would have to learn all the attributes listed with regard to a concept.

Step 3: Provide the student with useful verbal mediators

The teacher should ascertain the child's knowledge of the words used as attributes and attribute values and his knowledge of the relational words that are necessary. This step helps to see how the verbal and concept learning are related. The learning of certain names or labels (as verbal mediators) and specify type of verbal association facilities the students' learning of a concept.

Step 4: Provide positive and negative examples of the concept.

A positive example of a concept is one which contains the attributes of a concept. A negative example is one which does not contain one or mor more

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of the attributes. Positive examples of the concept bird are crow,parrot,pegion,cookoo, Negative examples are dog, cat, snake,fly, bat, bee. Use of positive and negative examples is a necessary condition for the learning of concepts.

The presentation of a mixed series of positive and negative examples is usually more effective than the presentation of a purely positive or a purely negative series. Presentation of only negative examples makes concept learning extremely difficult. As for number, enough positive examples to represent the range of attributes and attribute values of the concept should be presented. In the case of negative examples, at least, enough of these should be presented to eliminate irrelevant attributes which students are likely to include as part of the concept. Finally, direct experience or realistic examples are usually not preferable to simplified presentations of the concepts, such as line drawings, cartoons, diagrams and charts. These presentations help to achieve the effects of step 2, which directed to simplify the learning of the concept by focusing on its major attributes.

Steps 5: Present the examples in close succession or simultaneously

This step is concerned with the order in which the examples as a whole and the types of examples (positive and negative) are presented to the student. The learning condition is contiguity - the almost simultaneous presentation of the examples of the concept. Simultaneous presentation is better because the student does not have to rely upon memory or previous examples. In teaching the concept of dog,

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it is better to leave in view pictures of cats,birds,horses and dogs while presenting new pictures. By this miximization of contiguity and reduction of the information load on memory are taken care.

Step 6. Provide occasions for student responses and the reinforcement of these responses.

In concept learning reinforcement primarily provides informational feedback, which enables the learner either to separate positive and negative examples and to compose his list or to define the relationship of the various attributes. The primary purpose of reinforcement is to provide informational feedback to the student on the correctness of his responses. Since this feedback is crucial, any inconsistency, delay or failure to provide it will impair student learning. However, because the student knows which terminal behaviour he must acquire, he can to some extent monitor his own learning. Since reinforcement has motivational aspects, negative verbal feedback may impair concept learning by discouraging the student from making early guesses which can be confirmed. The teacher should remember to focus on the reinforcement of the students' responses and not on the student. The mode of the response should not be shifted, at least in the early learning of the concepts. It is quite possible, however, that the shift from spoken to written responses is less inhibiting than the shift from drawing to writing or writing to drawing.

Step 7: Assess the learning of the concept

In this step both contiguity and reinforcement are provided. This step emphasises generalisation, or the ability of the student to make the conceptual response to a new but similar pattern of stimuli. If the student is able to identify the new example of the concept, he has learned the concept. Tp provide reinforcement the student must be informed about the accuracy of his response. Several new positive and negative examples of the concept are to be presented and the student has to select only the positive examples. A small amount of practice of the definition, even when the students are not told how good the definition is, improves the quality of definitions. When the definition is difficult to formulate special training for formulation of concept definition should be imparted.

Finalisation of Instructional objectives

S.C. Panda

The whole educational system is directed towards certain aims such as utilitarian, cultural, vocational, all round development of the learner and the like. The school education programme is only a part of the total educational programme. However, it plays an important and even a vital role in the realisation of educational aims. 'What can the school education programme achieve?,' the question naturally arises.

It can achieve only a part of these broad educational aims which we refer to an objective. An objective is a pointer an end-view of the possible achievement in terms of what a student is to be able to do when the whole educational system is directed towards educational aims.

Objectives in Measurable Terms

On the other hand, educational objectives, bearing experiences, and evaluation procedures are the three instructive aspects of the educational process. Objectives play a key role in the instructional process. They serve as guide for both teaching and evaluation. Instructional objectives determine precisely and specifically what type of pupil performance is desired at the end of the instructional sequence. Educational objectives have been stated as broad and ultimate goals such as exercising the mental functions of reasoning, imagination, and memory. The objectives should be stated in terms of students' behaviour that can be observed and measured. Armed ~~with~~ a clear and specific list of teaching objectives, a teacher may consider the most appropriate procedures for evaluating progress made towards each objectives. He attempts to test what he has tried to teach by using techniques best suited to determine how well each objective is attained.

Classification of Instructional objectives

The instructional objectives have been classified basing on three major domains: cognitive, affective and psychomotor. It was based upon the assumpation that in the process of sharing of how information changes usually occur in the domains of cognitive, affective and psychomotor of the learner.

The objectives of the cognitive domain are phrased as descriptions of desired student behaviour - that is in terms of knowledge, understanding and abilities to be acquired. The large proportion of educational objectives fall into the cognitive domain. The affective domain includes objectives that emphasise interests, attitudes and values and the development of appreciation and adequate adjustment. Objectives in this domain are not stated very precisely and, infact, teachers do not appear to be very clear about the learning experiences which are appropriate to these objectives.

The psychomotor domain is concerned with physical, motor, or manipulative skills.

For further specifications of the taxonomy of educational objectives each of the three domains have been divided into a number of hierarchical categories of behaviours from simple to complex. For cognitive domain these six ascending levels are knowledge, comprehension, application, analysis, synthesis and evaluation. The five major categories of affective domain of the taxonomy of educational objectives are : receiving, responding, valuing, organisation, characterisation by a value or value complex. And finally seven major categories of psychomotor domain are: perception, set, guided response, mechanism, complex over response, adaptation and organisation.

While preparing instructional objectives it is possible to focus on different aspects of instruction. Our focus must on the behavioural changes(learning outcome) with the pupil which is based on the learning experiences provided to them.

Criterion for selecting behavioural objectives

In developing a list of objective for a particular course, however, the teacher is still faced with the problem of determining the adequacy of the final list of objectives. The following list of questions will serve as a criteria for this purpose.

1. Do the objectives include all important outcome of the course?
2. Are the objectives in harmony with the general goals of schools?
3. Are the objectives in social principles of learning ?
4. Are the objectives realistic in terms of the abilities of pupils and the time and facilities available ?

General Instructional objectives and specific learning outcome

In preparing a list of instructional objectives for a course of study we have two immediate goals in mind. One is to obtain as complete a list of objectives as possible. This is most likely to occur if we follow the procedures for selecting objectives described earlier. The other goal is to state the objectives so that they clearly indicate the learning outcomes that we expect from our instruction. The task of stating instructional objectives is simplified if we constantly keep in mind that we are making a list of intended outcomes of teaching learning situation.

1) We are not identifying subject matter content but the reaction pupils are to make to this content.
2) We are not listing the learning experiences of the pupils but the changes in pupils performance resulting from these experiences.
3) We are not describing what we intend to do during instruction but are making a list of the expected results of that instruction. Stating objective in terms of learning outcomes rather than learning process admittedly is easier said than done. If we

continually ask ourselves 'what should the pupils be able to do at the end of the course or unit of study, that they could not do at the begining. Then we find that the pupils terminal performance has almost automatically become the center focus. We are than in a much better position to state our instructional objectives in terms of learning outcomes.

A list of objectives for a course or unit of study should be detailed enough to clearly convey the intend of the instruction and get general enough to serve as an effective overall guide in planning for teaching and testing. This can be most easily accomplished by defining objectives in two steps.

1) Stating the general objectives of instruction as intended learning outcomes.

2) Listing under each objective a sample of specific type of performance that pupils are to demonstrate when they have achieved the objective. The procedure would result in statements of general instructional objectives and specific learning outcome like the following.

1. Understands scientific principles
    1.1 Describes the principles in his own words.
    1.2 Identifies examples of the principle
    1.3 States tanable hypothesis based on the principles
    1.4 Distinguish between two given principles.
    1.5 Explain the relationship between two given principles.

It is to be noted that the general objective starts right off with verb with precise wording directing to students outcome and free of course content. It should be unitary and realistic. Similarly it should be noted that specific learning outcome or specification is merely a sample of the many specific ways to realise the general objectives. In case of specification to each statement should begin with a verb indicating observable responses. The specific learning outcomes are free of course content, realistic,unitary and stated in precise terms. Action verb is a key element in stating the specific learning outcomes the selection and clarification of these verbs play an important role in obtaining a clearly defined set of instructional objectives. Ideally we would like each verb

(i) to clearly convey our instructional intent and

(ii) to precisely specify the pupil performance we are willing to accept as evidence that the general objectives has been attained.Unfortunately some verbs convey instructional intent well (e.g. identifies), other are more effective at precisely specififying the pupil responses to be observed (e.g.encircles,lables, underlines). Where it is necessary to choose between two types it would seen desirable to select than that most clearly convey instructional intent and if needed, to further clarify the expected pupil responses in one of the following ways.

(a) Add a third level of a specificity to the list of objectives. E.g.

1. Comprehend the meaning of written material
    - 1.1 Identifies the main thought in a passage
        - 1.1.1 Underlines the topic/sentence
        - 1.1.2 Selects the most appropriate title for the passage.
        - 1.1.3 Writes the main idea of the passage.

(b) Provide definitions of the action verb used in the specific learning outcomes. E.g.
Illustrations of how to clarify expected pupils responses for selected action verbs.

* Actionverb	Types of responses
Identify	Point to, touch, mark encircle, match, pick up.
Name	supply verbal label(orally or in writing)
Describe	supply a verbal account(orally or in writing)that gives the essential categories, properties and relationship.

Actionverb	Types of responses
Order	list in order, place in sequence, arrange, rearrange.
Construct	Draw, make design, assemble, prepare, build.
Demonstrate	perform a set of procedure with or without, a verbal explanation.

(c) Use sample test items to illustrate the intended outcomes.

* Sullivan, H.J. (1969) stated that these six action verbs and their synonyms encompass all cognitive learning outcomes in the school.

Summary of steps for stating Instructional Objectives

The final list of objectives for a course, or unit should include all important learning outcomes(e.g. knowledge, understanding, skills, attitude, and should be stated in a manner that clearly conveys what pupils are like at the end of the learning experience. The following summary of steps provides guidelines for obtaining a clear statement of instructional objectives.

I. Stating the General Instructional objectives

1. State each general objective as an intended learning outcome (e.g. pupils terminal performance)
2. Begin each general objective with a verb (e.g. knows, applies, interprets) "omit" the pupil should be able to ....
3. State each general objective to include only one general learning outcome (e.g. not knows and understands).
4. State each general objective at the proper level of generality (i.e. it should encompass a readily definable domain of responses) stating from eight to twelve general objectives will usually suffice.

5. Keep each general objective sufficiently free of course content so that it can be used with various units of study.

6. State each general objective so that there is minimum overlap with other objectives.

II. Stating the specific learning Outcomes

1. List beneath each general instructional objective a representative sample of specific learning outcomes that describes the terminal performance pupils are expected to demonstrate.

2. Begin each specific learning outcome with an active verb that specifies observable performance (e.g. identifies,describes).

3. Check to be sent that each specific learning outcome is relevant to general objective it describes.

4. Include a sufficient number of specific learning outcomes to describe adequately the performance of pupils who have attained the objectives.

5. Keep the specific learning outcomes sufficiently free from course content so that the list can be used for other units of the study.

6. Consult reference materials for the specific components of those complex outcomes that are difficult to define (e.g. critical thinking, scientific attitude, creativity).

7. Add a third level of specificity to the list of outcomes it needed.

Table - 1

Major Categories in the Cognitive Domain of the
Taxonomy of Educational Objective(Bloom,1956).

Descriptions of the Major Categories in the
Cognitive Domain

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1. Knowledge. Knowledge is defined as the remembering of previously learned material. This may involve the recall of a wide range of material, from specific facts to complete theories, but all that is required is the bring to mind of the appropriate information. Knowledge represents the lowest level of learning outcomes in the cognitive domain.

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2. Comprehension. Comprehension is defined as the ability to grasp the meaning of material. This may be shown by translating material from one form to another (words of numbers), by interpreting material (explaining or summarizing), and by estimating future trends (predicting consequences or effects). These learning outcomes go one step beyond the simple remembering of material, and represent the lowest level of understanding.

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3. Application. Application refers to the ability to use learned material in new and concrete situations. This may include the application of such things as rules, methods, concepts, principles, laws, and theories. Learning outcomes in this area require a higher level of understanding than those under comprehension.

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4. Analysis. Analysis refers to the ability to break down material into its component parts so that its organizational structure may be understood. This may include the identification of the parts, analysis of the relationships between parts, and recognition of the organizational principles involved. Learning outcomes here represent a higher intellectual level than comprehension and application because they require an understanding of both the content and the structural form of the material.

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5. Synthesis. Synthesis refers to the ability to put parts together to form a new whole. This may involve the production of a unique communication (theme or speech), a plan of operations (research proposal), or a set of abstract relations (scheme for classifying information). Learning outcomes in this area stress creative behaviours, with major emphasis on the formulation of new patterns or structures.

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6. Evaluation. Evaluation is concerned with the ability to judge the value of material (statement, novel, poem, research report) for a given purpose. The judgement are to be based on definite criteria. These may be internal criteria (organization) or external criteria (relevance to the purpose) and the student may determine the criteria or be given them. learning outcomes in this area are highest in the cognitive hierarchy because they contain elements of all of the other categories, plus value judgements based on clearly defined criteria.

Examples of General Instructional Objectives and Clarifying Verbs for the Cognitive Domain of the Taxonomy

Illustrative General Instructional Objectives	Illustrative Verbs for Stating specific Learning Outcomes
Knows common terms Knows specific facts Knows methods & Procedures knows basic concepts. Knows principles.	Defines, describes, identifies, labels, lists, matches, names, outlines, reproduces, selects, states.
Understand facts & Principles. Interprets verbal material Interprets charts and graphs Translates verbal material to mathematical formulas. Estimates consequences implied in data justifies methods and procedures.	Converts, defends, distinguishes, estimates, explains, extends, generalizes, gives examples, infers, paraphrases, predicts, rewrites, summarizes.

Applies principles to new situations. Applies theories to practical situation. Solve mathematical problems. Constructs charts and graphs. Demonstrates correct usage of a procedure.	Changes, computes, demonstrates, discovers, manipulates, modifies, operates, predicts, pre-pares, produces, relates, shows, solves, uses.
Recognizes unstated assumptions. Recognizes logical fallacies is reasoning Distinguishes between facts and inferences Evaluates the relevancy of data Analyzes the organizational structure of a work (art,music,writing)	Breaks down, diagrams, differentiates, discriminates, distinguishes, identifies, illustrates, infers, outlines, points out,relates, selects, separates, sub-divides.
Writes a well-organized theme. Gives a well-organized speed. Writes a creative short story(or poem) proposes a plan for an experiment Integrates learning from different areas into a plan for solving a problem Formulates a new scheme for classifying objects (or events or ideas)	Categorizes, combines, compilies, composes, creates, devises,designs, explains, generates,modifies,organizes, plans, rearranges,reconstructs, relates,reorganizes, revises,rewrites, summarizes,tells,writes.

Judges the consistency of written material. Judges the adequacy with which conclusions are supported by data. Judges the value of a work (art,music,writing) by use of internal criteria Judges the value of a work (art,music,writing) by use of external standards.	Appraise,compares,concludes, contrasts,criticizes, describes, discriminates, explains, justifies, interprets,relates,summarizes, supports.

Table -2

Major Categories in the Affective Domain of the Taxonomy of Educational Objectives(Krathwo,1964).

Description of the Major Categories in the Affective Domain

1. Receiving. Receiving refers to the student's willingness to attend to particular phenomena or stimuli(classroom activities textbook, music,etc.).From a teaching standpoint,it is concerned with getting,holding, and directing, the student's attention.Learning outcomes in this area range from the simple awareness that a thing exists to selective attention on the part of the learner. Receiving represents the lowest level of learning outcomes in the affective domain.

2. Responding. Responding refers to active participation on the part of the student. At this level he not only attends to a particular phenomenon but also reacts to it in some way.Learning outcomes in this area may emphasize acquiescence in responding(reads assigned material),willingness to respond (voluntarily reads beyond assignment), or satisfaction in responding (reads for pleasure or enjoyment). The higher levels of this category include those instructional objectives that are commonly classfied under interest; that is those that stress the seeking out and enjoyment of particular activities.

3. Valuing. Valuing is concerned with the worth or value a student attaches to a particular object, phenomenon, or behaviour. This ranges in degree from the more simple acceptence of a value (desires to improve group skills) to the more complex level of commitment (assumes responsibility for the effective functioning of the group). Valuing is based on the internalization of a set of specified values, but clues to these values are expressed in the student's overt behaviour. Learning outcomes in this area are concerned with behaviour that is consistent and stable enough to make the value clearly identfiable. Instructional objectives that are commonly classified under attitudes and appreciation would fall into this category.

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4. Organization. Organization is concerned with bringing together different values, resolving conflicts between them, and beginning the building of an internally consistent value system. Thus the emphasis is on comparing, relating, and synthesizing values. Learning outcomes may be concerned with the conceptualization of a value (recognizes the responsibility of each individual for improving human relations) or with the organization of a value system (develops a vocational plan that satisfies his need for both economic security and social service). Instructional objectives relating to the development of a philosophy of life would fall into this category.

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5. Characterization by a value complex. At this level of the affective domain, the individual has a value system that has controlled his behaviour for a sufficiently long time for him to have developed a characteristic life style. Thus the behaviour is pervasive, consistent and predictable. Learning outcomes at this level cover a broad range of activities but the major emphasis is on the fact that the behaviour is typical or characteristic of the student. Instructional objectives that are concerned with the student's general patterns of adjustment (personal, social, emotional) would be appropriate here.

Examples of General Instructional Objectives and Clarifying Verbs for the Affective Domain of the Taxonomy

Illustrative General Instructional Objective	Illustrative Verbs for stating Specific Learning Outcomes
Listens attentively Shows awareness of the importance of learning Shows sensitivity to social problems Accepts differences of race and culture Attends closely to the classroom activities.	Asks,chooses,describes, follows,gives,holds, identifies,locates,names, points to,selects,sits erect,replies, uses.
Completes assigned home- Obeys school rules Participate in class discussion Completes Laboratory work Volunteers for special tasks shows interest in Enjoys helping others.	Answers,assists,complies, conforms,discusses, greets, helps, labels,performs practices, presents, reads, recites,reports, selects, tells,writes.
Demonstrates belief in the democratic process. Appreciates good literature(art or music),Appreciates the role of science(or other subjects) in everyday life shows concern for the welfare of others.Demonstrates Problem-solving attitude Demonstrates commitment to social improvement.	Completes,describes,differentiates,explains,follows, forms,initiates,invites, joins,justifies,proposes, reads,reports,selects, shares,studies,works

Recognizes the need for balance between freedom and responsibility in a democracy, Recognizes the role of systematic planning in solving problem. Accepts responsibility for own behaviour. Understands and accepts own strengths and limitations. Formulates a life plan in harmony with his abilities interests, and beliefs.	Adheres, alters, arranges, Combines, compares, completes, defends, explains, generalizes, identifies, integrates, modifies, orders, organizes, prepares, relates, synthesizes,
Displays safety consciousness. Demonstrates self-reliance in working independently. Practices Cooperation in group activities. Uses objective approach in problem solving. Demonstrates industry and self-discipline Maintains good health habits.	Acts, discriminates, displays, influences, listens, modifies performs, practices, process, qualifies, questions, revises, serves, solves, uses, verities.

Table -3

A Classification of Educational Objectives in the Psychomotor Domain (Simpson, 1972).

Description of the Major Categories in the Psychomotor Domain

1. Perception. The first level is concerned with the use of the sense organs to obtain cues that guide motor ac-tivity. This category ranges from sensory stimulation (awareness of a stimulus), through cue selection (selecting task-relevant cues), to translation (relating cue perception to action in a performance).

2. Set. Set refers to readiness to take a particular type of action. This category includes mental set (mental readiness to act), physical set (Physical readiness to act) and emotional set (willingness to acat). Perception of cues serves as an important prerequisite for this level.

3. Guided Response. Guided response is concerned with the early sages in learning à complex skill. It includes imitation (repeating an acat demonstrated by the instructor) and trial and error (using a multiple-response approach to identify an appropriate response). Adequacy of performance is judged by an instructor or by a suitable set of criteria.

4. Mechanism. Mechanism is concerned with performance acts where the learned responses have become habitual and the movements can be performed with some confidence and proficiency.Learning outcomes at this level are concerned with performance skills of various types,but the movement patterns are less complex than at the next higher level.

5. Complex Overt Reponse.--Complex Overt Response is concerned with the skillful performance of motor acts that involve complex movement patterns. Proficiency is indicated by a quick,smooth,accurate performance, requiring a minimum of energy.This category includes resolution of uncertainity (performs without hesitation) and automatic performance (movements are made with ease and good music control). Learning outcome at this level include highly coordinated motor activities.

6. Adaptation.Adaptation is concerned with skills that are so well developed that the individual can modify movement patterns to fit special requirement or to meet a problem situation.

7. Origination. Origination refers to the creating of new movement patterns to fit a particular situation or specific problem. Learning outcomes at this level emphasize creativity based upon highly developed skills.

Example of General Instructional Objectives and Clarifying Verbs for the psychomotor Domain.

Illustrative General Instructional Objectives	Illustrative Verbs for Stating Specific Learning outcomes
Recognizes malfunction by sound of machine. Relates taste of food to need for seasoning Relates music to a particular dance step.	Chooses,describes,detects, differentiates, distinguishes, identifies,isolates, relates, selects, separates.
Knows sequence of steps in Varnishing wood Demonstrates proper bodily stance for batting a ball Shows desire to type efficiently.	Begins, displays,explains, moves, proceeds, reacts, responds, shows,starts, volunteers.
Performs a golf swing as demonstrated Applies first aid bandage as demonstrated Determines best sequence for preparing a meal.	Assembles,builds,calibrates, constructs, dismantles, displays,disects,fastens, fixes,grinds,heats, manipulates,measure, mends mixes, organizes, sketches.
Writes smoothly and legibly Sets up laboratory equipment Operates a slide projector Demonstrates a simple dance step.	(Same list as for Guided Response).
Operates a power saw skillfully Demonstrates correct form in swimming Demonstrates skill in driving an automobile Performs skillfully on the Violin Repairs electronic equipment quickly and accurately.	(Same list as for Guided Response)
Adjusts tennis play to counteract opponent's style Modifies swimming strokes to fit the roughness of the water.	Adapts,alters, changes, rearranges,reorganizes, revises, varies.
Creates a dance step Creates a musical composition Designs a new dress style.	Arranges,combines,composes, constructs,creates,designs, originates.

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